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Dr.S.Thangavelu
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Dr.T.L.Ratnakumari
Executive Editor

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TOPIC OF INTEREST - "VACCINOLOGY I"

DRUG PROFILE

Journal Office and address for communications: Dr. S.Thangavelu, Editor-in-Chief, Indian Journal of Practical Pediatrics, 1A, Block II, Krsna Apartments, 50, Halls Road, Egmore, Chennai - 600 008. Tamil Nadu, India. Tel.No. : 044-28190032 E.mail : ijpp_iap@rediffmail.com

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VACCINOLOGY I

SCIENCE AND PRACTICE OF VACCINE SCHEDULING

**** Puneet Kumar***
***** Vipin M Vashishtha***

Abstract: *Vaccination is the most successful and cost-effective health intervention in human history. The success of vaccination programs stands on three pillars namely safe and effective vaccines, high population coverage and optimal scheduling. The scheduling of any vaccine is not straight forward. It is affected by immunological, epidemiological, programmatic factors and the dynamic interactions among these factors in any given population at any given time. This article describes the science and practice behind this scheduling and how this scheduling is different for an individual child as recommended by Indian Academy of Pediatrics and for the community at large, as represented by the National immunization chart (Universal Immunization Program).*

Keywords: *Scheduling vaccines, Immunization chart, Universal immunization program.*

Vaccines represent one of the most successful and cost-effective health interventions in human history which have helped in reducing both morbidity and mortality from Vaccine Preventable diseases (VPD). Global vaccination programs save up to 2-3 million lives annually as per WHO.[1] While smallpox has been eradicated with vaccination program, other infections like poliomyelitis are also on the verge of the same. Three key pillars to achieve such phenomenal outcomes are efficacious vaccines which are of public health importance, right scheduling (timing) of the vaccines to have greatest impact and good coverage of the target population so as to achieve herd immunity (herd immunity helps by protecting the non-vaccinated, immunologically naive and immunocompromised individuals by reducing the percentage of vulnerable hosts to a level below the transmission threshold). At an individual level, an appropriate scheduling of vaccines gives the best chance of protection against VPDs for a long time (preferably life long) and with minimum number of visits to a health care facility. This article will briefly describe the science behind the scheduling of vaccines from individual as well as community perspective.

* Physician,
Kumar Child Clinic,
Dwarka, New Delhi,

** Director and Consultant Pediatrician,
Mangla Hospital & Research Center,
Bijnor, India.

email: vipinipsita@gmail.com

The immunization schedule

The immunization schedule depicts the optimal timing (age) of administration of one or more vaccines.

The factors which are taken into consideration while deciding the optimal scheduling of any vaccine are: 1) Immunological factors, 2) Epidemiological factors 3) Programmatic and logistic factors 4) Synergizing between various factors 5) Purpose of immunization (Community vs Individual protection).

1. Immunological factors: These factors are the minimum age at which the said vaccine has good efficacy, the number of doses / boosters required and spacing between the doses.

a) The minimum age at which a vaccine is efficacious: This depends further on at least 2 factors: the type of immune response elicited by the vaccine and the corresponding immunological maturity of the recipient. For example, the polysaccharide antigens are T-cell independent. Further, the "marginal zones" of the spleen/ lymph nodes are immature and induction of germinal centers (GCs) is also limited and delayed in early life leading to poor antibody response by B-cells in the absence of "help" from T-cells. Thus, the polysaccharide vaccines like typhoid and pneumococcal polysaccharide vaccines are not immunogenic in early life and minimum age of administration is 2 years when the immune system is more mature.[2] However, conjugation of these polysaccharide antigens with proteins improves antigen presentation and induces T-cell response too. Hence the conjugated polysaccharide vaccines are effective in early life. Second important factor affecting the minimum age for optimal vaccine efficacy is the degree of inhibition of immune response by the maternal antibodies that are

passively transferred to the baby. The inhibitory influence of maternal antibodies on infant B cell responses affects all vaccine types, although its influence is more marked for live attenuated viral vaccines that may be neutralized by even minute amounts of passive antibodies.[3] This inhibition is because of the binding of maternal antibodies to their specific epitopes at the antigen surface, competing with infant B cells and thus limiting B cell activation, proliferation and differentiation.[2] Hence, vaccines like measles and measles-mumps-rubella (MMR) are not administered in early infancy. At the same time, Bacillus Calmette-Guerin (BCG) vaccine elicits T-cell response is effective even at birth, since infant T-cell responses are largely unaffected (or even enhanced)[3] by antibodies of maternal origin. Oral polio vaccine (OPV) and rotavirus vaccines are also hardly affected by maternal antibodies.[4]

b) Number of doses required: It in turn depends on the type of vaccine, age of the recipient and interval between doses. Type of the vaccine is an important factor and in general, inactivated vaccines fail to generate GCs, thereby limiting the induction of memory responses and high affinity long life plasma cells. Therefore, multiple doses are needed to provide primary immunity, e.g. pertussis vaccine. In contrast, live attenuated vaccines induce more sustained antibody response presumably through antigen persistence in the host (the attenuated virus multiplies in the host), therefore, a single dose may suffice to provide adequate primary immunity as in BCG and yellow fever vaccines.[2] In some other live attenuated vaccines like measles and varicella, a small percentage of babies fail to seroconvert (primary vaccine failure) and second dose is recommended so that seroconversion occurs in a very high percentage of population.[3] However, repeated boosters are not needed for these unlike for DTP vaccine where the immunity wanes off with time. Live attenuated vaccines like OPV require multiple doses because of poor take possibly due to high prevalence of gut pathogens. Age of recipient is also important since immunological maturation increases with age and older babies have better and early induction of the germinal centers leading to better antigen-specific B-cell proliferation and differentiation.[3] Thus the number of doses required to immunize a baby with conjugate vaccines decreases as the age advances. Similarly, DTP vaccine requires 5 doses when started at 6 weeks of age while only 3 doses are required if it is started at one year of age.

c) Spacing between doses: Interval between the doses also affects the number of doses. For primary immunization with IPV, 3 doses are required if it is started at 6 weeks with one-month interval between the doses, but only 2 doses of IPV are sufficient, if it is started after 8 weeks of age and two-month interval is kept between 2 doses. Between two primary doses of any vaccine, minimum interval of 4 weeks is always necessary since this allows development of successive waves of antigen-specific primary responses without interference.[2] Similarly, interval between last dose of primary series and its booster is never kept less than 4 months as this allows affinity maturation of memory B-cells and thus, higher secondary responses. How frequently the boosters are to be repeated depends on the duration of the immune response elicited after the 1st booster. Faster waning of immunity necessitates more frequent boosters.

Further, the scheduling is affected in individual cases under special circumstances based on immunological factors operating therein. For example, when both a live vaccine (like measles or varicella vaccine) and antibodies such as IVIG for ITP or Kawasaki disease, are to be given around same time, the two should be separated by enough time interval so that the antibody does not affect the replication of live attenuated virus which is essential for optimal immune response. If the live vaccine is given first, it is necessary to wait at least 2 weeks (i.e. an incubation period) before giving the antibody. If the antibody is given before a dose of MMR or varicella vaccine, it is necessary to wait until the antibody has waned (degraded) before giving the vaccine to reduce the chance of interference by the antibody.[4]

The necessary interval between an antibody containing product and MMR or varicella containing vaccine (except zoster vaccine) depends on the concentration of antibody in the product but is always 3 months or longer and can be as long as 11 months.[5] The same holds true for blood, FFP, intravenous immunoglobulin (IVIG) infusion since they contain antibodies. Since the inactivated antigens (like rabies, hepatitis B or tetanus) are generally not affected by circulating antibody, they can be administered before, after, or at the same time as the antibody. Some live vaccines like oral Ty21a typhoid vaccine, rotavirus vaccine, yellow fever vaccine and live attenuated influenza vaccine are also not affected much by the circulating antibody and can be administered before, concurrent with or after administration of any immunoglobulin, hyperimmunoglobulin or IVIG.[5]

In children on immunosuppressive drugs, vaccination needs to be delayed until the drugs are tapered for optimal immune response. A child who has undergone bone marrow ablation needs to be revaccinated with all age-appropriate vaccines as all the memory B-cells are destroyed with the therapy.[5]

2. Epidemiological factors: Susceptibility for infection, age-specific incidence rates and severity/ mortality associated with the infection are important considerations while scheduling any vaccine.[2,4] Measles and varicella vaccines beautifully explain these factors. Both these vaccines, being live-attenuated viral vaccines, are subjected to significant inhibition by maternally derived antibodies. However, measles has high incidence in infancy in developing countries like India and is associated with significant morbidity and mortality. Hence, measles vaccine is scheduled at 9 months of age, though the vaccine efficacy is not optimal at this age. The idea is to prevent as many infections as possible. If the vaccine is scheduled after 1 year of age, many infants would have already suffered from it by that time. To make up for the sub-optimal efficacy, another dose is scheduled at 15 months of age. In the setting of an outbreak, the measles vaccine is given to infants as young as 6 months, again to save as many infants as possible during the outbreak. However, the efficacy is very poor at this age. Thus, this dose is not counted, and regular doses are given at 9 months and 15 months of age, as scheduled. At the same time, in developed countries where the incidence is much less, measles vaccine is not recommended at 9 months of age. Vaccination against measles begins at 12-15 months of age with optimal efficacy. Varicella vaccine, which is also a live-attenuated viral vaccine which can be inhibited by maternal antibodies is not scheduled in infancy, since the incidence of varicella as well as morbidity is not as much as with measles. Thus, vaccination against varicella begins after 15 months of age even in developing countries like India. Yet another example is that of H. influenzae and pneumococcal conjugate vaccines which do not provide very long-term immunity. Still, repeated boosters are not scheduled unlike tetanus vaccine since the incidence of H.influenzae and pneumococcal infections is rare in otherwise healthy children above 5 years of age.

The scheduling of vaccines is not fixed, but dynamic. With change in local epidemiology of the disease, the schedule needs to be updated accordingly. For example, with control of poliomyelitis, the OPV is being phased out as a part of strategy of polio end game. Most countries which have eliminated polio have already discontinued OPV. Similarly, with good coverage of vaccine and decrease in incidence of measles in the country, its schedule would also be updated with dropping of the dose that is currently scheduled at 9 months of age.

3. Programmatic and logistic factors: To improve compliance and reduce visits to health care facility, the vaccines are scheduled and adjusted in line with the opportunity to provide other health interventions during the visits for vaccination. Sometimes, availability of a combination vaccine forces us to consider scheduling the vaccine accordingly. For example, although the best schedule for hepatitis B vaccine immunologically is 0-1 month-6months, it is often given at birth and then combined with DTP/Hib/IPV vaccine at 6-10-14 weeks or 8-12-16 weeks since it reduces the number of visits and pricks, thus decreasing the drop outs. Interactions among different vaccines also need to be considered. The different vaccines scheduled at the same age should not be interfering with each other's immune response when given on the same day. Moreover, cost-effectiveness analysis, overall financial burden, availability, adequate vaccine supply, transport, storage (cold chain) and trained staff/ human resources are also considered, especially when mass vaccination and/ or National immunization schedule is being formulated.[6]

4. Synergizing between various factors: Integrating the various factors while designing an immunization schedule, especially at the national level is not easy. Issues like achieving protective immune response prior to the age when children are most vulnerable versus inducing optimal immune response causes greatest concern. Starting late might induce a higher response but miss the vulnerable age. For example, if the first 3 doses of DTP/ H.influenzae/ Hepatitis B/ IPV are given at 2, 4 and 6 months, they elicit better immunological response (late beginning with wider inter-dose intervals). However, since significant number of babies get infected in early infancy, many developing countries including India schedule these vaccines at 6/10/14 weeks to immunize babies early, but this is at the cost of some decrease in efficacy. It is a trade-off. In developed countries with lower incidence of these infections in infancy, 2/4/6-month or 2/3/5-month schedule is followed.

Need to relook at the existing EPI schedule?

In the recent years many experts have suggested that the decades old 6/10/14 week be relooked urgently even in developing countries like India,[7,8] since increasing the interval between doses can increase seroconversion of pertussis and OPV vaccines by upto 10-15%, although the difference may not be significant for diphtheria and tetanus toxoids.[9] Further, vaccines like H. influenzae, pneumococcal and rotavirus vaccines (RVVs) are also being scheduled at 6/10/14 weeks because of programmatic and logistic reasons while this schedule is less optimal immunologically.

In a recent systematic review and meta-analysis[10] of interventions designed to increase oral vaccine efficacy or

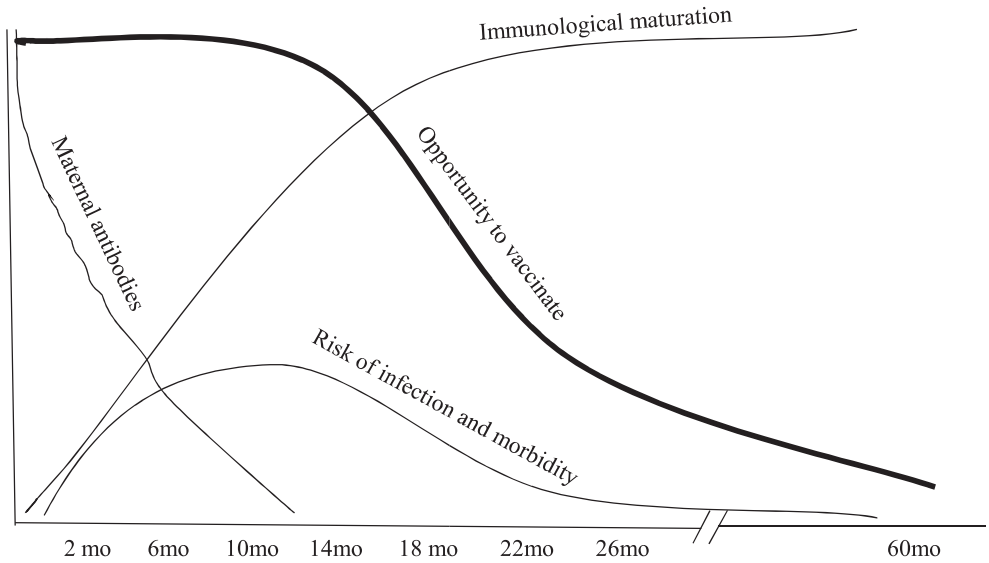

Fig.1a. Interplay of various factors in scheduling of measles vaccine

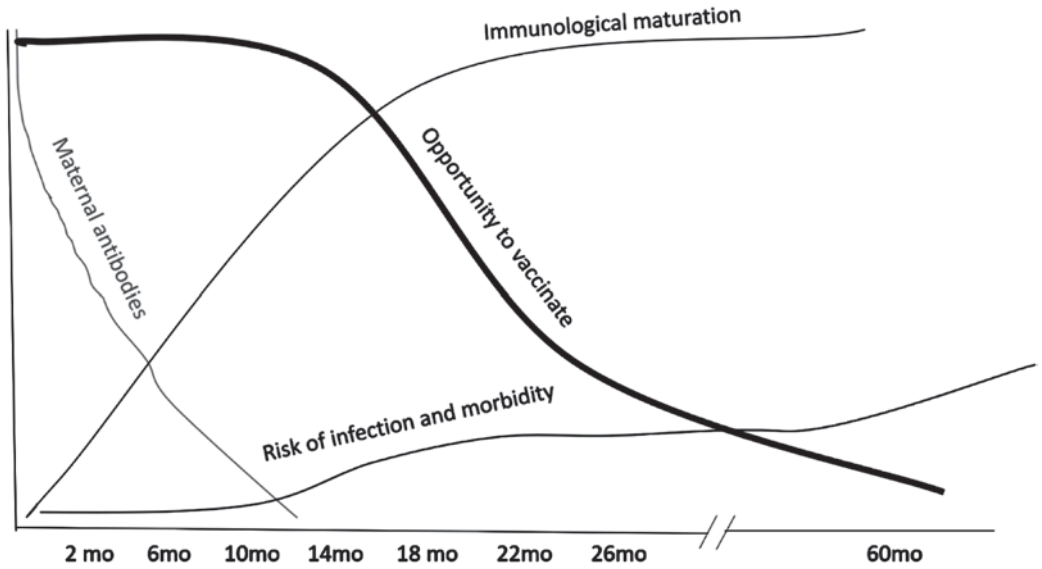

Fig.1b. Conceptual respresentation of interplay of various factors affecting scheduling of varicella vaccine

immunogenicity in lower and lower middle-income countries (LICs and LMICs), the researchers included 87 studies for qualitative synthesis and 66 for meta-analysis. They assessed 22 different interventions for OPV, RVV, oral cholera vaccine, and oral typhoid vaccines. The researchers concluded that most strategies did not improve oral vaccine performance. However, delaying RVV and reducing OPV valence (monovalent or bivalent compared to trivalent OPV) were the only interventions that were found effective in improving their performance. There was no evidence of effect for anthelmentics anthelmintics, antibiotics, probiotics, zinc, vitamin A, withholding breastfeeding, extra doses, or vaccine buffering.[10] Hence, slight delay in the scheduling of RVV may have beneficial effects on its ultimate performance.

Shifting to 2/4/6 mo schedule will increase efficacy of all these vaccines. Moreover, with vaccine being recommended at 6 month of age would be a good opportunity to give advice on complementary feeding at the right age which can be a game-changer in preventing babies falling in the trap of malnutrition at this crucial juncture. Yet another advantage could be decreasing the gap between the third dose of these primary vaccines and first dose of measles containing vaccine. The increased gap is thought to be one factor in high dropout rate of vaccination program in late infancy that is seen currently.

Another example to demonstrate the interplay of various factors is scheduling of measles and varicella vaccines. Fig.1a and 1b display these interactions. Both are live attenuated viral vaccines and are inhibited by maternally derived antibodies. Since the risk of infection with measles (and associated morbidity) in developing countries is higher in infancy, its vaccine is scheduled at 9 months of age (even at the cost of less efficacy), but varicella vaccine is scheduled after 15 months of age since the risk of infection (and associated morbidity) is much less in infancy. Other factors like maturation of immune system and the nature of antigen also impact scheduling of vaccines.

Purpose of immunization (Community vs individual protection): This is another important factor that has a big influence on the scheduling of vaccines. When the vaccines are being scheduled for an individual child, the schedule is designed so as to provide best protection with utmost safety and cost effectiveness. The immunization chart that is recommended by the Indian Academy of Pediatrics (IAP) is the one that is recommended for office practice with an average child-client of office practitioners.[11] On the other hand, the National Immunization Schedule is designed to reduce child mortality and morbidity on a national level using the most cost-effective vaccines. Although cost, efficacy and safety are important considerations in both the charts, the priorities change. The IAP chart would give priority to safety, efficacy and cost in this order while national immunization chart, the order is cost, efficacy and then safety.[4] Table I gives the key differences between IAP and national schedule.

Nevertheless, it is also a fact that what is not in the best interests of the individual cannot be in the best interests of the community and what is in the best interests of the community is also in the best interests of the individual. Therefore, the private health-care and public health programs including vaccination schedules should be complementary and not contradictory regarding immunological basics, ethics and epidemiology.

Conclusions

Optimal scheduling of vaccines to be based on immunological, epidemiological and programmatic/ logistic factors so as to deliver optimal results in prevention of disease and reduction of associated morbidity and mortality in the individual child or at community level, as the case may be. None of the immunization schedule charts is rigid and needs to be updated with changing epidemiology, gain in insight/ data and availability of new and newer vaccines.

Table I. Key differences between National and IAP schedule[12]

Attributes	National immunization schedule	IAP immunization schedule
Focus	Community: Vaccination in public health is in the best interests of community	Individual child; Vaccination in health-care is in the best interests of each child
Need	Determined by the epidemiology and disease burden in the community	Determined by the risk (probability of disease) to an individual child
Objective	To control a set of infectious diseases from the community Control = reduce incidence and monitor reduction	To protect the individual who is vaccinated
Ownership	Government	Consulting physician/pediatrician or individual health facility
Volume	Large	Small
AEFI	Mainly coincidental and programmatic since large volume is used	Mainly vaccine reactions and coincidental
Funding	By the government or international donor agencies	Individual parents
Logistic issues	Major determinants	Not so important
Considerations	Cost first, efficacy next, safety last	Safety first, efficacy next, cost last

Points to Remember

- ***Vaccine schedule is planned based on immunological, epidemiological, programmatic factors and the dynamic interactions among these factors.***
- ***National Immunization schedule is mainly focussed on the community, because responsibility of public health is in the best interest of community.***
- ***IAP Immunization schedule is focused on the individual child, because vaccination in health-care is in the best interest of each child.***
- ***Though the objectives are slightly different, the private health-care and public health programs including vaccination schedules should be complementary and not contradictory regarding immunological basics, ethics and epidemiology.***
- ***The scheduling of vaccines is not fixed, but is a dynamic one depending on local epidemiology of the disease, gain in insight/ data and availability of the newer vaccines.***

References

1. World Health Organization. Immunization. Available online: https://www.who.int/news-room/facts-in-pictures/detail/immunization. Accessed on November 5, 2020.
2. Pemde HK. Basic immunology. In: Advisory Committee on Vaccines and Immunization Practices, Indian Academy of Pediatrics. IAP Guidebook on Immunization 2018-2019. Balasubramanian S, Shashtri DD, Shah AK, Pallab Chatterjee, Harish Pemde, Shivananda S, Vijaya Kumar Guduru (eds), 3rd Edn, Jaypee Brothers Medical Publishers, New Delhi, 2020; pp14-27.
3. Siegrist CA. Vaccine immunology. In: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines. 6th edn. Philadelphia: Saunders Elsevier; 2013; pp14-33.
4. Chatterjee P. Scheduling of vaccines. In: Advisory Committee on Vaccines and Immunization Practices, Indian Academy of Pediatrics. "IAP Guidebook on Immunization 2018-2019, 3rd Edn, Jaypee Brothers Medical Publishers, New Delhi, 2020; pp84-92.
5. Shastri DD. Immunization in Special Situations. In: Advisory Committee on Vaccines and Immunization Practices, Indian Academy of Pediatrics. IAP Guidebook on Immunization 2018-2019, Balasubramanian S, Shashtri DD, Shah AK, Pallab Chatterjee, Harish Pemde, Shivananda S, Vijaya Kumar Guduru (eds), 3rd Edn, Jaypee Brothers Medical Publishers, New Delhi, 2020; pp405-432.
6. Kumar P, Vashishtha VM. The issues related to introduction of a new vaccine in National Immunization Program of a developing country. J Pediatr Sci 2010; 5:e45.
7. Mittal SK, Mathew JL. Expanded program of immunization in India: time to rethink and revamp. J Pediatr Sci 2010; 5:e43.
8. Vashishtha VM, Kumar P. 50 years of immunization in India: progress and future. Indian Pediatr 2013; 50(1): 111-118.
9. Edwards KM, Decker MD. Pertussis vaccines. In:Plotkin SA, Orenstein WA eds. Vaccines 4th edn, Philadelphia, Saunders 2004; pp471-528.
10. Church JA, Parker EP, Kirkpatrick BD, Grassly NC, Prendergast AJ. Interventions to improve oral vaccine performance: a systematic review and meta-analysis. Lancet Infect Dis 2019; 19(2):203-214.
11. Balasubramanian S, Shah A, Pemde HK, Chatterjee P, Shivananda S, Guduru V, et al. Indian Academy of Pediatrics (IAP) Advisory Committee on Vaccines and Immunization Practices (ACVIP) Recommended Immunization Schedule (2018-19) and Update on Immunization for Children Aged 0 Through 18 Years. Indian Pediatr 2018; 55:1066-1074.
12. Vashishtha, VM. Scheduling of Vaccines. In: Gupta P, Menon PSN, Ramji S, Lodha R, Editors. Textbook of Pediatrics for Postgraduates, 2ndEdn: Jaypee Bros, New Delhi, 2017; pp1053-1059.

CLIPPINGS

A brief review of the mRNA vaccines.

These type of vaccines provide a modern approach to vaccination. Unlike traditional vaccines, mRNA vaccines trigger an immune response by giving cells the molecular sequence needed to make an associated protein. Due to their high potency, rapid development capacity, and potential for low cost manufacturing and safe administration, mRNA vaccines present a promising alternative to conventional vaccine approaches. Although mRNA is genetic material, there is no risk of genetic side-effects, as RNA vaccines cannot alter the genome.

Bari NA, A Review of the Cellular Biomechanics, Development and Safety of mRNA Vaccines. Ann Arbor, 1001:p48109.

VACCINOLOGY I

IMMUNOLOGY OF VACCINES - AN UPDATE

**** Baldev S Prajapati***
***** Rajal B Prajapati***

Abstract: *Immunology is a complex subject but understanding the basic functions of the immune system is useful in order to know how the vaccines work, the basis of recommendations for their use, various immunization schedules, combination of vaccines, modifications in reference to epidemiology of the disease, special situations, etc. It is interesting to know how the immune system reacts to live vaccines, inactivated vaccines, polysaccharide and conjugated vaccines. The functioning of antigen presenting cells, dendritic cells, germinal centres and marginal zones in spleen and lymph nodes is very complex. T cell dependent and T cell independent immune responses to different vaccines decide the quality of antibodies and duration of protection. They further decide the number of primary doses and need for boosting. Due to the presence of immune memory, there is no need to restart the entire schedule in case of interrupted vaccinations. The primary and secondary immune responses explain the lag period, types of immunoglobulins produced and duration of protection. The influence of extremes of age, malnutrition, genetic and environmental factors on the immunology of vaccination is a fascinating study.*

Keywords: *Vaccination, Immunology.*

Although immunology is a complex subject, understanding the basic functions of the immune system is useful in order to learn how vaccines work, the basis of recommendations for their use, various immunization schedules, combinations of vaccines, modifications in reference to epidemiology of the disease and special situations.

* HOD & Professor of Pediatrics,
GCS Medical College Hospital and Research Centre,
Ahmedabad.

** Former Professor of Pediatrics,
AMC MET Medical College,
V.S. General Hospital, Ahmedabad.

email: baldevprajapati55@gmail.com

Immunization is the process of inducing immunity against a specific disease - this maybe passive through administration of preformed antibody containing preparations or active by administering a vaccine to stimulate the immune system to produce a prolonged humoral and/or cellular immune response. Passive immunity offers transient protection against the disease. for e.g., immunoglobulins and specific or hyperimmune immunoglobulins. Active immunization is achieved by administering vaccines by different routes like oral, nasal or by injection (subcutaneous, intradermal or intramuscular routes).

Vaccines are of two types - live attenuated and inactivated. Live attenuated vaccines are produced by modifying a disease producing organism in the laboratory. The resulting vaccine strain retains its ability to replicate and to produce immunity, but loses its pathogenicity to produce the disease. Inactivated vaccines are composed either of the whole organism or fractions of it. Fractional vaccines are either protein based or polysaccharide based. Protein based vaccines are toxoids, subunits or subvirion products. Polysaccharide antigens are chemically linked to protein to make more potent vaccines.

The immune response to various vaccines differs and depends upon several factors.

Innate and adaptive immunity

'Innate immunity' comprises of the skin and mucosal barriers, phagocytes like neutrophils, monocytes, macrophages and natural killer (NK) cells. It comes into play immediately on entry of the pathogen and is nonspecific. 'Adaptive immunity' is provided by B lymphocytes in the form of humoral or antibody mediated immunity and T lymphocytes in the form of cellular or cell mediated immunity. The innate immune system triggers the development of adaptive immunity by presenting antigens to the B lymphocytes and T lymphocytes. Adaptive immunity takes time to evolve and is pathogen specific (Fig.1).[1]

Immune system is not mature at birth and in young infants. Maternal antibodies that are transferred transplacentally and immunological components in breast

milk provide some protection during early childhood. After birth, the baby comes in contact with microbes which gradually activate the immune system. B cells form the most important component of the immune system in the body. They are produced by the liver in foetal life and later mature in the bone marrow in humans. In other species these cells mature in an organ celled "bursa of Fabricius", hence these lymphocytes are called B cells. On activation by an antigen contained in micro organisms and vaccines, the B cells proliferate and get converted to plasma cells which in turn produce antibodies. For effective production of antibodies, B cells need the help of T helper cells.[2]

Naive T lymphocytes originate in the thymus and enter the blood stream. After entering the blood stream, they pass through the spleen and lymph nodes where they encounter thousands of antigen presenting cells (APCs). Here the T cell receptors bind to an antigen presented by a major histocompatibility complex (MHC) molecule and get activated. They subsequently receive additional costimulation signals driving them to proliferate and acquire killing (mainly CD8 + T cells) or supporting (mainly CD4 + T cells) functions, memory cells etc.[2] T - cell activation requires two signals, T - cell receptors (TCR) and costimulation. Ultimately, they are transformed into antigen specific T - cells.

B cells have Ig surface receptor which binds with the appropriate antigen present on the infective pathogen. The processed antigen stimulates the B cells to mature into antibody secreting plasma cells and generate IgM. T helper 2 (Th 2) cell leads to switch in the production from Ig M to Ig G, Ig A or Ig D. The B cells can directly respond to antigen and process the antigen, but T cells do not react with the antigen directly unless processed and presented by special cells called antigen presenting cells.[2]

Antigen presenting cells and dendritic cells

APCs are the cells that capture antigens, process them into small peptides, display them at their surface through MHC molecules and provide costimulation signals that act synergistically to activate antigen specific T cells. APCs include B cells, macrophages and Dendritic cells (DCs), although only DCs are capable of activating naive T cells (T cells being exposed to antigens for the first time) (Fig.2).[3]

The major role of DCs is to identify dangers which is done by the special receptors on the APCs known as Toll - Like Receptors (TLRs). Vaccine antigens are taken up by immature DCs activated by the local inflammation, which then provide the signals required for their migration to draining lymph nodes. During the migration, DCs mature and their surface expression of molecules changes. Simultaneously, antigens are processed into small fragments and displayed at the cell surface in the grooves of MHC (HLA in humans) molecules. As a rule, MHC class I molecules present peptides from antigens that are

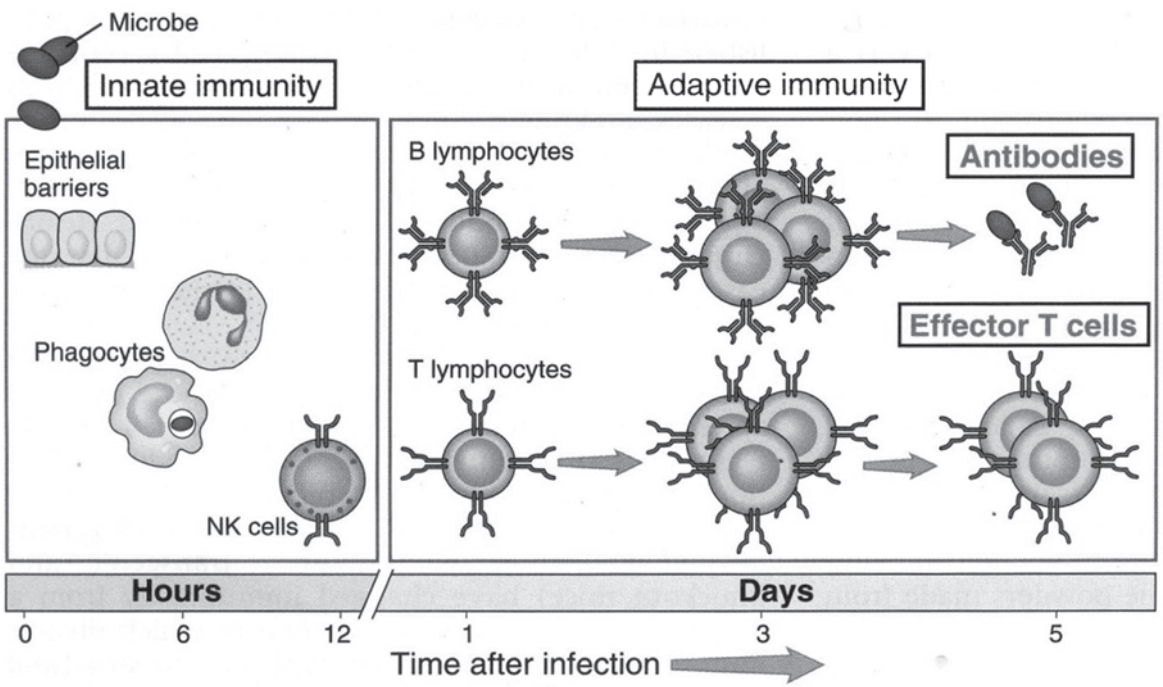

Fig.1. Innate and adaptive Immunity

Source: Adapted from Vashishtha VM, Kalra A, Thacker N (Eds.) FAQ on Vaccines and Immunization Practices. New Delhi: Jaypee Brothers;2011.

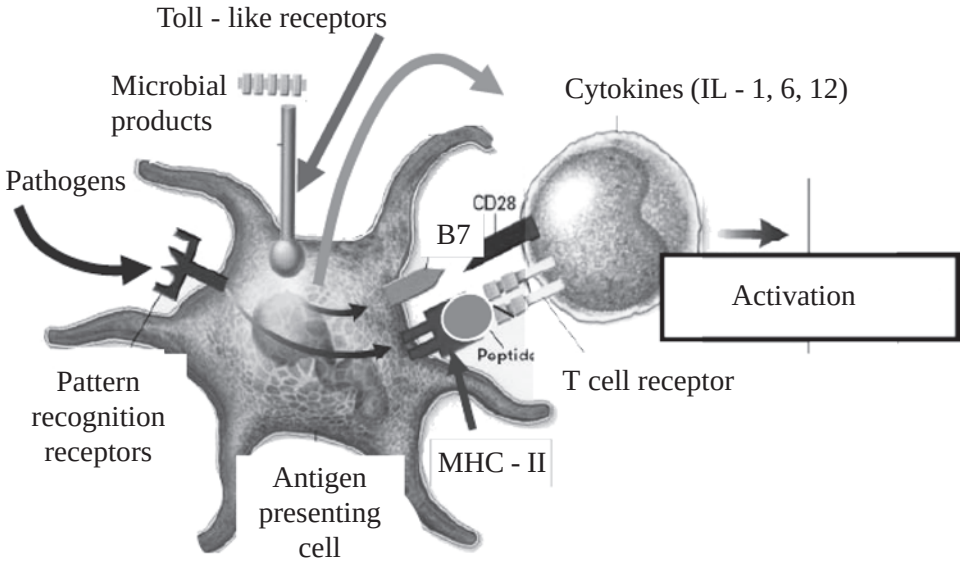

Fig.2. Schematic presentation of a dendritic cell and its activation of pathogens

Source: Siegrist CA. Vaccine Immunology. In: Plotkin SA, Orenstein W, Offit P (Eds). Vaccines, 5th edition. Saunders Elsevier; 2008: 17-36.

produced within infected cells, whereas phagocytosed antigens are displayed on MHC class II molecules. Thus, mature DCs reaching the T cell zone of lymph nodes display MHC - peptide complexes and high level of costimulation molecules at their surface. CD4 + T cells recognize antigenic peptides displayed by MHC class II molecules, whereas CD8 + T cells bind to MHC class I peptide complexes. Antigen specific T cell receptors may only bind to specific MHC molecules (HLAA2), which differ among individual populations. Consequently, T cell responses are highly variable within a population.[3]

Immune responses elicited by vaccines

Live viral vaccines efficiently trigger activation of the innate immune system, probably through pathogen associated signals like viral RNA, allowing their recognition by pattern recognition receptors (PRR) type of - TLRs. Following injection, viral particles rapidly disseminate throughout the vascular network and reach their target tissues. This mechanism is very similar to natural infection, including the initial mucosal replication stage for vaccines administered through oral and nasal routes. Following the administration of a live viral vaccine and its dissemination, DCs are activated at multiple sites, migrate toward the corresponding lymph nodes and launch multiple foci of T cell and B cell activation. This explains the generally higher immunogenicity of live versus inactivated vaccines. The strongest antibody responses are generally elicited by live vaccines that better activate innate immune responses and stimulate better induction of adaptive immune effectors.[1-4]

Inactivated vaccines frequently need to be formulated with adjuvants of which aluminium salts are specifically potent enhancers of antibody responses. Therefore, adjuvants are included in a majority of currently available inactivated vaccines. The adjuvant also causes local deposition of an antigen which is slowly released and in turn extends the duration of B cell and T cell activation, as well as the preferential induction of IL-4 by aluminium exposed macrophages. Very few inactivated vaccines induce high and sustained antibody responses after a single vaccine dose even in healthy individuals. Therefore, primary immunization schedules usually require at least two vaccine doses, repeated at minimal interval of 4 weeks to generate successive waves of B cell and germinal centre responses. Occasionally, these priming doses may be combined into a single dose with doubling of the strength of an antigen. However, antibodies elicited by primary immunization by inactivated vaccines eventually wane in due course of time.[1-4]

Germinal centres and marginal zone

Germinal centres (GCs) are dynamic structures that develop in spleen and lymph nodes in response to antigenic stimulation and get resorbed after a few weeks. GCs contain a monoclonal population of antigen-specific B cells that proliferate and differentiate through the support provided by follicular DCs and helper T cells. Immunoglobulin class switch recombination, affinity maturation, B cell selection and differentiation into plasma cells or memory B cells essentially occur in GCs.[2,3]

Marginal zone is the area between the red pulp and the white pulp of the spleen. Its major function is to trap particulate antigens from the circulation and present it to lymphocytes.[2,3,5]

An 'epitope' also called as antigenic determinant, is that part of an antigen that is recognized by the immune system, specifically by antibodies, B cells or T cells. The part of an antibody that recognizes the epitope is called as 'paratope'.

Antibody affinity refers to the tendency of an antibody to bind to a specific epitope at the surface of an antigen. The avidity is the sum of the epitope - specific affinities for a given antigen. It directly relates to its function.

Toll - like receptors (TLRs) and their role in vaccine immunogenicity

Toll - like receptors are a class of proteins playing an important role in the innate system. They are single membrane-spanning non-catalytic receptors that recognize structurally conserved molecules derived from the microbes. Once these microbes have breached the physical barriers like skin or intestinal tract mucosa, they are recognized by TLRs which activate immune cell responses. TLRs are a family of ten receptors (TLR1 to TLR10) that present at the surface of many immune cells, which recognize pathogens through conserved microbial pattern and activate innate immunity on detecting danger. They are a type of pattern recognition receptor (PRR) and recognize molecules that are broadly shared by pathogens but distinguishable from host molecules, collectively referred to as pathogen-associated molecular patterns (PAMPs). TLRs together with interleukin-1 receptors form a receptor super family, which are known as, the 'interleukin-1 receptor / toll like receptor / toll like receptor super family'. All members of this family have in common so called TIR (Toll-IL-I receptor) domain. The TLRs appear to be one of the most ancient, conserved components of the immune system.[2,3]

T-cell dependent and T-cell independent immune responses

Certain antigens, primarily proteins, induce both B cell and T cell stimulation leading to what is called T cell dependent immune response. Infants of 6 weeks of age and above are capable of mounting a T cell dependent response which usually results in higher titres of IgG and is long lasting. It also shows good booster effects with repeated doses.[2,3]

T cell independent response being only B cell mediated, is not possible in children below 2 years of age. It is mainly IgM type response with low titres. The response is short lasting and has no booster effect on repeat doses. IgA is not produced and hence there is no local mucosal protection with this type of antigens, while in case of T cell dependent response, IgA is produced which provides mucosal protection and eradication of the carrier state. Bacterial polysaccharide (PS) antigens of Streptococcus pneumoniae, Neisseria meningitides, Hemophilus influenzae B and Salmonella typhi are examples of T cell independent antigens which can be made T cell dependent by the technique of conjugation.[2-3,6-7] Such conjugated PS vaccines (conjugated Vi typhoid, Hib, pneumococcal and meningococcal vaccines) are effective in children less than 2 years of age.

Immune responses at cellular level

The first steps after immunization

After an injection, vaccine antigens attract local and systemic DCs, monocytes and neutrophils. These activated cells change their surface receptors and migrate along lymphatic vessels to the draining lymph nodes where the activation of T and B lymphocytes takes place. In case of inactivated vaccines, there is only local and unilateral lymph node activation. For live vaccines there is multifocal lymph node activation due to microbial replication and dissemination. Consequently, the immunogenicity of inactivated vaccines is lower than the live vaccines. Inactivated vaccines require adjuvants which improve the immune response by producing local inflammation and recruiting DCs to the site of injection. The site of administration of inactivated vaccines is of importance; the intramuscular route which is well vascularised and has a large number of patrolling DCs is preferred over subcutaneous route. The site of administration of vaccine is usually not of significance for live vaccines. Finally, due to focal lymph node activation, multiple inactivated vaccines may be administered at different sites with insignificant immunologic interference. This phenomenon may occur with multiple live vaccines unless they are given on the same day or at least 4 weeks apart.[2-4,8]

• Immune responses of T cell independent antigens at the cellular level

These antigens are non-proteinaceous, polysaccharides in nature. On being released from the injection site, these antigens reach the marginal zone of the spleen or lymph node and bind to specific immunoglobulin surface receptors of B cells. In the absence of antigen specific T cell help, B cells are activated, undergo proliferation and differentiation into plasma cells without undergoing affinity maturation in GCs.

The antibody response sets in 2 to 4 weeks following immunization. It is mainly of IgM type with low titres of low affinity IgG. The half life of plasma cells is short and antibody titres decline rapidly. Additionally, the PS antigens are unable to evoke an immune response in children less than 2 years of age due to immaturity of marginal zones. As PS antigens do not induce GCs, bonafide memory cells are not elicited. As a result, subsequent re-exposure to the same PS results in a repeated primary response that follows the same kinetics in previously vaccinated as in naive individuals. Revaccination with certain PS such as group C meningococcus may even induce lower antibody responses than the first immunization. This phenomenon is called hyporesponsiveness whose molecular and cellular basis are not yet fully understood.[1-2,9-10]

• Immune responses of T cell dependent antigens at the cellular level

T cell dependent antigens include protein antigens which may consist of either pure protein (hepatitis B, hepatitis A, toxoids, HPV) or conjugated protein carrier with PS antigens (Hib, pneumococcal, meningococcal, Salmonella Vi antigen). The first response to these antigens is similar to PS antigens. However, the antigen specific helper T cells that have been activated by antigen bearing DCs trigger some antigen specific B cells to migrate toward follicular dendritic cells (FDCs), initiating GC reaction. In GCs, B cells receive additional signals from follicular T cells and undergo massive clonal proliferation, switch from IgM toward IgG and IgA, undergo affinity maturation and differentiate into plasma cells secreting large amounts of antigen specific antibodies.

Most of the plasma cells die at the end of GC reaction and thus the decline in antibody level is noted 4-8 weeks after vaccination. However, a few plasma cells exit spleen and lymph nodes and migrate to survival niches located in the bone marrow, where they survive through signals provided by supporting stromal cells and this results in prolonged persistence of antibodies in the serum.[2,3,7]

• Memory B cell Response

Memory B cells are those B lymphocytes that are generated in response to T dependent antigens, during GC reaction, in parallel to plasma cells (PC). They persist there as resting cells until re-exposed to their specific antigens. They then readily proliferate and differentiate into plasma cells secreting large amount of high affinity antibodies that may be detected in the serum within a few days after boosting. These cells also undergo affinity maturation in GC.

These cells are generated during T cell dependent responses including GC responses. These cells are resting cells that do not produce antibodies. Memory B cells undergo affinity maturation in 4 to 6 months. A minimal interval of 4 to 6 months is required for optimal affinity maturation of memory B cells. They rapidly differentiate into antibody secreting plasma cells upon re-exposure to antigen. Memory B cells differentiate into PCs that produce higher affinity antibodies than the primary plasma cells. As plasma cells and memory responses are generated in parallel in GCs, higher post-primary antibody titres reflect stronger GC reactions and generally predict higher secondary responses. During induction, a lower antigen dose at priming results in inducing B cell differentiation away from PCs and towards memory B cells.[1-3]

Implications of immune memory on immunization programs are as follows.[2-4]

- Immunization schedule should never be started 'all over again' regardless of duration of interruption.
- Certain immunization schedules may not require boosters if exposure provides regular natural boosters.
- Boosters may not be needed where re-activation of immune memory by offending pathogen is sufficiently rapid and effective to interrupt microbial invasion e.g. Hepatitis B.
- Interval between primary and booster doses should be a minimum of 4 to 6 months.

Immune response to live vaccines

The live vaccines induce an immune response similar to that seen with protein vaccines and natural infection. However, take of live vaccines is not 100% with the first dose. Hence more than one dose is recommended with most live vaccines. Once the vaccine is taken up, immunity is robust and lifelong or at least for several decades. This is because of continuous replication of the organism that serves as a constant source of the antigen. The second dose of the live vaccine is mostly for primary vaccine failure (no uptake of vaccine) and not for secondary vaccine failures (decline in antibodies over time).[2,3]

Determinants of intensity and duration of immune responses

The nature of the antigen is the primary determinant. Generally speaking, live vaccines are superior (BCG and OPV are exceptions) to protein antigens which in turn are superior to PS antigens. The presence of adjuvants improves immune responses to inactivated vaccines.

Higher the antigen concentration, better is the immune response (e.g. hepatitis B). The immune response can be improved with increasing the number of doses and increasing the interval between the doses. Long lasting plasma cells in the bone marrow and reactivation of memory B cells provide longer duration of protection against invading organisms in an immunized individual.

Ideally vaccination at 0, 1 and 6 months is the best immunization schedule. The first two doses are for induction (priming) and the long gap between the second and third dose allows affinity maturation of B cells for booster and memory response. Extremes of age and disease conditions lower immune responses.[2-4]

Primary and secondary immune responses

When an antigen is introduced for the first time, the immune system responds primarily after a lag period of 7 to 10 days. It is called the "primary response". Subsequently, upon re-introduction of the same antigen, there is no lag period and the immune system responds by producing antibodies immediately and this is called as "secondary response". However, there are some differences in both these responses. Primary immune response is short lived, has a lag phase, predominantly of IgM type and the antibody titres are low, whereas the secondary immune response is almost immediate, without a lag phase, antibody titres are very high, persisting for a long time

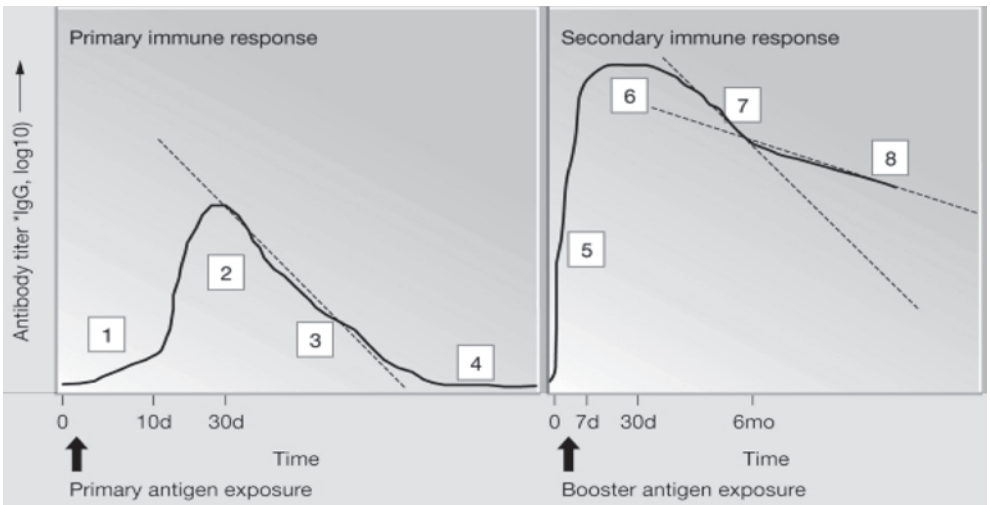

Fig.3. Correlation of antibody titres to various phases of the vaccine response

1. The initial antigen exposure elicits an extrafollicular response.

2. That results in the rapid appearance of low IgG antibody titres. As B cells proliferate in germinal centres and differentiate into plasma cells, IgG antibody titres increase up to a peak value usually reached 4 weeks after immunization.

3. The short life span of these plasma cells results in a rapid decline of antibody titres.

4. Which eventually return to baseline levels.

5. In secondary immune responses, booster exposure to antigen reactivates immune memory and results in a rapid (< 7 days) increase of IgG antibody titre.

6. Short - lived plasma cells maintain peak Ab levels during a few weeks after which;

7. Serum antibody titres decline initially with the same rapid kinetics as following primary immunization. Long - lived plasma cells that have reached survival niches in the bone marrow continue to produce antigen - specific antibodies.

8. Which then decline with slower kinetics.

Note: This generic pattern may not apply to live vaccines triggering long - term IgG antibodies for extended periods of time.

Source: Siegrist CA. Vaccine Immunology. In: Plotkin SA, Orenstein W, Offit P (Eds). Vaccines, 5th edn. Sounders Elsevier;2008:17-36).

and predominantly of Ig G type. Fig.3 describes the background developments at the cellular level and interactions of - B cells, memory B cells and T cells at the follicular level in a lymph node. The secondary response is mainly due to booster response and is seen with vaccines that work on a 'prima-boost' mechanism inducing T cells such as conjugate vaccines. On the other hand, non - conjugate, PS vaccines mainly induce primary response and the repeat dose also produces another wave of primary response and does not act as booster since it does not induce T cells.[2-3,7]

Limitations of immunization in the young

Maternal antibodies inhibit antibody responses to most vaccine antigens in young infants. IgG antibodies are actively transferred through the placenta from the maternal to the foetal circulation. Upon vaccination, maternal antibodies bind to their specific epitopes at the antigen surface, competing with the infant B cells and thus limit B cell activation, proliferation and differentiation. The inhibitory influence of maternal antibodies on infant B cell responses affects all vaccine types, although the effect is more so with live attenuated vaccines. Hence, antibody responses elicited in early life are short lasting.[11-13]

Magnitude of response to all vaccines in early life is characterized by age dependent limitations. Antibody responses to PS antigens are not elicited during the first two years of life. It is due to several reasons like slow maturation of the spleen marginal zone, limited expression of CD21 on B cells and limited availability of the complements.[11]

Although maternal antibodies interfere with the induction of antibody response in infants, some degree of priming effect is seen. The priming effect is in the form of induction of memory B cells. This indicates that limited amount of vaccine antigens may be sufficient for priming of memory B cells, but not capable of GC activation. It is a well known fact that maternal antibodies do not inhibit T cell responses in infants. During post natal life, maternal antibodies gradually wane and their inhibitory influence on infant's immune response declines.[12-13]

Other factors which influences immune response

- Genetic factors: The capacity of antigen epitopes to associate with a large number of MHC molecules increases the likelihood of responses in the population. MHC restriction may limit T cell responses. Gene polymorphisms in molecules critical for B and T cell activation and differentiation are likely to affect antibody responses. T cell response differs significantly between individuals and populations due to genetic variations of MHC molecules (HLA A2).
- Environmental factors: Likely to play a role, but yet to be identified
- Nutritional factors: Compromised immune response following vaccination in a child suffering from severe acute malnutrition is a well-known fact.

Immune memory and need for boosters

Immune memory is seen with live vaccines and protein antigens due to generation of memory B cells which are activated on repeat vaccination or natural exposure. There is no need to restart the entire schedule in case of missed vaccinations because of this phenomenon. Activation of immune memory and generation of protective antibodies usually takes 5 to 7 days. Diseases which have incubation periods shorter than this like Hib, tetanus, diphtheria and pertussis require regular booster doses to maintain protective antibody levels. However, diseases like Hepatitis A and Hepatitis B do not need regular booster doses as the long incubation period of the diseases allows for activation of immune memory cells.[2-4]

Correlates of vaccine - mediated immunity

A given marker that is measurable, whether the antibody or a cellular component elicited in response to a vaccine that confers protection against a disease is termed as "correlate of protection". Conventionally, because of the relative ease of measurement, it is a specific antibody in the serum. Measurement of cellular components is difficult, invasive and highly cost intensive. The antibody correlates can be absolute which are directly protective. e.g. Hib (0.15 μg/ml), hepatitis B (10 mIU/ml), or surrogates (indirect markers), e.g. Varicella (> 5gpElisa units) and Rota (serum IgA). Vaccines for diseases like HPV, however do not have established correlates till now. The correlates of protection are important to confirm immunity and to compare vaccines. Therefore, correlates of protection should be standardized and replicable.[14]

Points to Remember

- ***Understanding basics of the immune system is useful to learn how vaccines work, basis of recommendation of various immunization schedules, combination of vaccines, modifications in reference to epidemiology of disease and special situations.***
- ***T cell dependent and T cell independent immune responses to various vaccines decide the quality of antibodies and duration of protection.***

- ***Because of immune memory there is no need to restart the entire vaccine schedule in case of an interruption. This phenomenon also decides the need for booster doses.***
- ***The primary and secondary immune responses explain lag period, types of immunoglobulins and duration of protection.***
- ***Extremes of age, malnutrition, genetic and environmental factors also play a role in immunological response to vaccines.***

References

1. Vashishtha VM, Kalra A, Thacker N. (Eds) FAQs on Vaccines & Immunization Practices. 1st edn. New Delhi: Jaypee Brothers. 2011; pp3-23.
2. Siegrist CA. Vaccine Immunology. In: Plotkin SA, Orenstein WA, Offit PA (Eds.) Vaccines. 5th edn. China: Saunders 2008; pp17-36.
3. Kamat D, Madhur A. Vaccine Immunology. In: Vashishtha VM (Ed). IAP Textbook of Vaccines. New Delhi: Jaypee Brothers, 2013; pp 25-36.
4. Plotkin SA. Vaccines, vaccination and vaccinology. J Infect Dis. 2003;187:1349-1359.
5. MacLennan ICM, Toellner KM, Cunnigham AF, Serre K, Sze DM, Zúñiga E, et al. Extrafollicular antibody responses Immunol Rev 2003; 194:8-18.
6. Lee CJ, Lee LH, Lu CS, Wu A. Bacterial polysaccharides as vaccine immunity & chemical characterization. Adv Exp Med Biol 2001; 491:453-471.
7. Pemde S. General Aspects of Vaccination. In: Balasubramanian S, Shashtri DD, Shah AK, Pallab Chatterjee, Harish Pemde, Shivananda S, Vijaya Kumar Guduru (Eds.) IAP Guidebook on Immunization. 3rd edn. New Delhi: Jaypee Brothers Medical Publisher 2020;pp14-27.
8. Hong Kong Measles Committee. Comparative trial of live attenuated measles vaccine in Hong Kong by intramuscular and intradermal injection. Bull World Health Organ 1967; 36:375- 384.
9. Plotkin SA. Vaccination against the major infectious diseases. CR Acad Sci III 1999; 322:943-951.
10. Kobrynski LJ, Sousa AO, Nahmias AJ, Lee FK. Cutting edge: Antibody production to pneumococcal polysaccharides requires CD1 molecules and CD8 + T Cells. J Immunol 2005; 174:1787-1790.
11. Siegrist CA. Neonatal and early life vaccinology. Vaccine 2001; 19:3331-3346.
12. Siegrist CA. Mechanisms by which maternal antibodies influence infant vaccine responses: Review of hypotheses and definition of main determinants. Vaccine 2003; 21:3406-3412.
13. Rowe J, Poolman JT, Macaubas C, Sly PD, Loh R, Holt PG, et al. Enhancement of vaccine specific cellular immunity in infants by passively acquired maternal antibody. Vaccine 2004; 22:3986-3992.
14. Plotkin SA. Vaccine correlates of vaccine induced immunity. Clin Infect Dis 2008; 47:401-409.

CLIPPINGS

Staphyloccal vaccine – is there a road ahead?

In this comprehensive review of the scientific evidence based study of S. aureus infections in humans, the authors believe that several conclusions can be reached. First, there are protective and detrimental immune responses in humans that correlate with clinical outcomes. Second, S. aureus toxins, especially super antigens and pore forming toxins disrupt host innate and adaptive immune responses that are important in protective immunity. Third, specific naturally-generated or vaccine-induced anti-S. aureus toxin antibodies are associated with improved clinical outcomes. Fourth, while the preponderance of studies suggest that toxins make S. aureus infections more severe and anti-toxin antibodies reduce the severity, the correlation is not 100%. This is likely due to genetic variability amongst humans in terms of responses to toxins and in the expression of the various toxins by the infecting S. aureus strain. Hence, the complexity of interactions between S. aureus and the host will be significant when considering multiple toxins, multiple genes involved in the human response, and variable levels of antitoxin antibody production. It is likely that the efficacy of a multivalent anti-toxin vaccine will have some variability in comparative outcomes as not every control subject will respond in the same way. Finally, the host response and toxins are likely different among the anatomical sites of infection and future vaccine effects must take these tissue-specific responses into account. Ultimately, many factors from human data should be considered in the future development an effective anti-S. aureus toxin vaccine along with measured and reasonable expectations to provide a better therapeutic approach to combat invasive S. aureus infections

Miller LS, Fowler VG, Shukla SK, Rose WE, Proctor RA. Development of a vaccine against Staphylococcus aureus invasive infections: Evidence based on human immunity, genetics and bacterial evasion mechanisms, FEMS Microbiology Reviews, 2020; 44(1):123-153.

VACCINOLOGY I

DIPHTHERIA, PERTUSSIS, TETANUS VACCINES

** Sanghamitra Ray*
*** Harish K Pemde*

Abstract: *DPT vaccine is one of the oldest vaccines mankind was gifted with. Over time newer vaccines like DTaP were introduced with lesser side effects but shorter lasting immunity. Both IAP and Govt of India endorse DTwP vaccine because of its greater efficacy. In 2018, TT vaccine was replaced with Td for vaccination at 10, 16 years in UIP schedule in India. Adolescent and adult vaccination now include one single dose of Tdap. Pregnant women are also now recommended to have a single dose of Tdap followed by Td as a routine immunization. Pertagen is a newly developed monovalent acellular pertussis vaccine containing genetically inactivated Pertussis Toxin. Boostagen (TdaPBioNet) is produced with genetically inactivated recombinant B. pertussis component. Both these vaccines are licensed in Thailand and further studies on these vaccines are going on in many developed countries. There are few newer combination hexavalent vaccines containing DPT, Hib, Hep-B and IPV which are also equally efficacious and have the potential to replace the routine vaccines in near future.*

Keywords: *DPT vaccine, Immunization, Newer pertussis vaccines.*

Diphtheria, pertussis, and tetanus combination vaccine (DPT) is one of the very old vaccines and is part of the immunization program of almost all countries. This article briefly reviews the DPT vaccines and also provides some information on newer DPT vaccines.

Diphtheria is one of the most devastating infectious diseases mankind has probably seen. After introduction of DPT vaccine in 1940, the global disease burden of diphtheria has gone down drastically. However, many countries of Africa, Middle-East and Latin America where vaccination coverage is suboptimal, face sporadic surges in diphtheria cases. The number of cases reported to WHO has declined from nearly 100,000 cases in 1980 to 2,500 cases in 2015.[1] A similar declining trend has also been observed in India; however, India is burdened with majority of diphtheria cases reported globally. During 2001-2015 almost half of the diphtheria cases reported globally were from India and 9622 cases were reported in 2019.[2] In the South East Asian region, India contributes to the maximum number of diphtheria cases.

Pertussis is another serious disease of childhood and most of the mortality occurs in infantile age group. This disease causes epidemics every 3 to 5 years worldwide. Pertussis vaccine has been a part of expanded programme of immunization (EPI) since 1974 causing almost 15-fold reduction in cases. A shift in the age distribution of pertussis towards older age groups including adolescents and adults has been reported in recent years in many developed countries particularly with the use of acellular pertussis components.

Recently there has been surge of cases with 132,754 cases reported globally and 11,875 cases from India in 2019.[2,3] Resurgence can be attributed to greater surveillance and sensitive diagnostic tools.

Tetanus: In the last twenty years the global mortality rates for tetanus declined by more than 30% with an annual rate reduction of 8.9% for neonatal tetanus. India has also performed brilliantly in reducing neonatal tetanus. It is a major milestone for India that it achieved its goal of elimination of maternal and neonatal tetanus in May 2015. Only 35 cases of neonatal tetanus cases were reported in India in 2019.[2] But in countries with inadequate immunization coverage, cases of neonatal and postneonatal tetanus are still prevalent and without proper care these children will have almost 100% mortality.

Whole cell pertussis vaccine was prepared from suspensions of whole killed organisms in 1914. Subsequently diphtheria and tetanus vaccines were developed in the year 1926 and 1938 respectively. In 1948 pertussis vaccine was further combined with diphtheria and tetanus toxoids formulating DPT vaccine. The conventional

* Assistant Professor of Pediatrics,
Chacha Nehru Bal Chikitsalaya, Delhi.

** Director Professor, Department of Pediatrics,
Lady Hardinge Medical College and
Kalawati Saran Children's Hospital, New Delhi.

email: harishpemde@gmail.com

DPT vaccine contains killed whole cell pertussis bacilli known as DTwP vaccine. Standard amount of diphtheria toxoid in the vaccine ranges from 20 to 30 Lf whereas amount of tetanus toxoid varies from 5 to 25 Lf. For diphtheria and tetanus vaccines the potency and amount of toxoid are recorded in International Units (IU) and in Limits of Flocculation (Lf) respectively. Insoluble aluminum salt is used as the adjuvant in this vaccine. Protective titre for diphtheria is >0.1 IU/ml and for tetanus is >0.01IU/ml. High maternal antitoxin antibody levels affect the immune response of the infant, leading to a lower immune response after the first 2 doses of diphtheria-containing vaccines, though most infants develop protective levels of antibody after completion of the full 3-dose primary series. The proven efficacy of DTwP vaccines is around 46% to 92%.[4] Most modern DPT vaccines have efficacy closer to 90% as that of diphtheria and tetanus components is around 90% and the pertussis component, around 70%.

DTaP vaccine

Despite the fact that DTwP has good protective efficacy after primary vaccination, its widespread implementation led to more attention to its associated side effects. As side effects of DTwP led to reduced acceptability among general population, acellular component of pertussis (aP) was combined with diphtheria and pertussis components, making a new alternative - DTaP. This was developed in Japan and is currently being used in many countries globally including India. This vaccine contains purified antigens such as pertussis toxin, pertactin, filamentous hemagglutinins and fimbrial hemagglutinins. Many different combinations and amounts of these antigens in different formulations are available. At present there is no consensus regarding the antigenic composition of an ideal pertussis vaccine but vaccines containing three or more antigenic components are more immunogenic than those containing lesser components. However, the latter vaccines are also quite effective.

Whole cell and acellular pertussis vaccines - Comparison

Initially it was presumed based on studies that DTaP and DTwP are almost similar in terms of duration of protection and efficacy. But now it is a proven fact that whole cell pertussis (wP) vaccines have greater efficacy than acellular pertussis (aP) vaccines. Studies after several outbreaks in United States, Australia and United Kingdom have so far concluded that the change from whole cell vaccines to acellular vaccines have contributed to the recent increase in the pertussis cases. Recent data from the US and Australia have suggested reduced duration (about 2 years) of vaccine-induced immunity after aP vaccination as compared to that after wP vaccination. Following concerns about the global resurgence of pertussis, a detailed review was done by WHO in the year 2014.[5] Data from nineteen high and middle income countries provided no evidence of outbreak of pertussis. In most countries increasing numbers of pertussis cases were attributed to naturally occurring cyclical pattern. The causes of increased reported cases include higher disease awareness, improved surveillance and the enhanced diagnostic sensitivity. However, there is evidence that a true resurgence had occurred in 5 of the 19 countries reviewed, 4 of which were exclusively using aP vaccines. The observed increase in cases in the 5th country, which used wP vaccine, was related to factors other than the vaccine type.[6]

Recently another factor has been implicated in favor of wP vaccine. As per the findings of animal studies, there is an important role of both Th1 and Th17 cells in the immune response to natural infection and to DTwP vaccine. The aP vaccines induce higher Th2, but lower Th1 and Th17 responses and are less effective in clearing the pertussis organisms and preventing transmission.[7] This is probably explained by some mucosal immunity following wP vaccines. The aP vaccines do not provide mucosal immunity. Thus, aP vaccine recipients are protected from disease but not from colonization and they can spread the disease to their contacts. This is the reason nasal pertussis vaccines are being developed that can provide immunity in the mucosa of the nose as well as lungs.

Routine vaccination schedule in India

According to the universal immunization programme (UIP) DPT vaccine is administered in primary immunization along with hepatitis B and Hemophilus influenzae type b called pentavalent vaccine. It is administered at 6, 10 and 14 weeks and catch up vaccination can be given with pentavalent vaccine till 1 year of age. First booster of DPT is given at 16 to 24 months while second booster is recommended at 5-6 years of age.[8] In August 2018, as per the recommendation of NTAGI (National Technical Advisory Group on Immunization) Government of India decided to replace Tetanus Toxoid vaccine provided at 10 and 16 years under the UIP schedule with Tetanus and adult diphtheria (Td) vaccine.[9]

Immunogenicity of booster vaccines in 4 to 6 year old children

A Canadian study evaluated the immunogenicity of lower content pertussis and diphtheria containing vaccine

Adacel (Sanofi) (Tdap) with a higher content pertussis and diphtheria vaccine Quadracel (DTaP-IPV) where children received three primary doses and 18-month booster prior to immunization at 4-6 years of age. The majority of children in both groups had a four-fold rise in pertussis antibodies one month after immunization.[10]

It has been seen that seroprotection in subjects ten years after immunization against diphtheria does not differ significantly between those vaccinated with pediatric dose (DTaP) or reduced dose (Tdap) product. The Tdap vaccine is highly immunogenic for diphtheria toxoids regardless of prior vaccination history (2+1 and 3+1 schedules).[11] However, full dose diphtheria containing vaccines (DTwP or DTaP) vaccines are preferred for 4-6 years old booster dose. Tdap can be used only when other standalone vaccines are not available or feasible or not desired by parents for fear of adverse events.

The second booster dose at 4-6 years is important and should be given preferably using a full dose vaccine with or without other vaccines in combination. The preferences for 4-6 years boosters are (1) standalone DPT vaccine, (2) DPT vaccine with other vaccines indicated at the age (e.g. DTaP/IPV) (3) DPT vaccine with other vaccines even when these are not indicated between 4-6 years (although additional vaccine doses do not generally harm, the additional doses should be avoided) and (4) Tdap when nothing else is available or when there is a history of severe local reactions to the previous doses of DPT or when parents insist on 'painless vaccine'. However, pros and cons of these options should be discussed with the parents before arriving at a decision.

Universal immunization programme (UIP) and Indian Academy of Pediatrics (IAP) schedule of DPT containing vaccines in India (Table I).

Adverse reactions

Concerns regarding side effects of pertussis containing vaccines have always been a challenge for achieving universal vaccine coverage. Even though severe adverse events are rare, side effects may consist of temperature in excess of 40.5 °C (0.3% of vaccine recipients), febrile seizures (8 per 100,000 doses) or hypotonic-hyporesponsive episodes (0-291 per 100,000 doses). During primary immunization, severe adverse events (anaphylaxis or encephalopathy) are similar in occurrence both with DTaP and DTwP vaccines. Seizures, persistent crying, hypotonic-hyporesponsive episodes and fever more than 40°C are less commonly reported with DTaP.[12] In adults, rates of local reactions are more frequently observed with booster doses containing 12 Lf compared with 5 or 2 Lf of diphtheria toxoid.[13] Such observations have resulted in the recommendation to provide low-dose diphtheria toxoid (Td) for immunization of individuals aged 7 years and above. Available data suggest that both tetanus and diphtheria toxoid contribute to the reactogenicity of Td and DT.[14]

There is often controversy regarding use of paracetamol along with DPT vaccination either as prophylaxis or as a treatment of fever, the most common side effect after DPT. But now there is ample evidence that paracetamol does not impair the immunological response to DPT containing vaccines.[15] Government of India also recommends paracetamol after vaccination for

Table I. Comparison of IAP and UIP schedule of DPT

	IAP (ACVIP)	UIP GOI)
Primary immunization	3 doses (DTwP/DTaP)	3 doses (DTwP)
First booster at 1½ yrs of age	DTwP/DTaP	DTwP
Second booster at 4-6 yrs of age	DTwP/DTaP	DTwP
10 year booster	Tdap	Td
16 year booster	Td	Td
Diphtheria vaccines - 5 full doses and 2 reduced doses Tetanus vaccines - 7 full doses Pertussis vaccines - 5 full doses of wP/aP (plus one more ap in ACVIP schedule)		

fever and reduction of pain and swelling at the injection site[16] as these minor side effects reduce vaccine acceptance in society. Routine paracetamol administration is not necessary.

Concomitant administration of vaccines containing DTaP or DTwP and other childhood vaccines does not interfere with the antibody response to any of the involved antigens.[17] Co-administration of DPT containing vaccines with BCG, conjugate pneumococcal or meningococcal vaccines, hepatitis B, injectable and oral polio, measles, measles and rubella, rotavirus, varicella and Hib vaccines is safe and does not result in decreased immunogenicity.[17]

Contraindications to DPT vaccine

Absolute contraindications to all DPT containing vaccines are history of anaphylaxis or encephalopathy not attributable to any underlying cause and onset within 7 days of vaccination. Progressive neurological disease is a relative contraindication to the first dose of DTwP vaccine. Other side effects such as hypotonic hyporesponsive episodes, persistent inconsolable crying, and fever more than 40.5^0C with previous vaccination only need precautions during the next dose of vaccination as recurrence of these symptoms with subsequent doses are rare.

Catch-up vaccination (Table II)

Newer pertussis vaccines

The ongoing resurgence in pertussis incidence in many of the high income countries is likely due to faster waning of immunity and increased colonization in individuals vaccinated with aP vaccine as compared to those vaccinated with wP. Pertussis and its toxins have been investigated by scientists for over a century, yet its pathogenesis is somewhat elusive.

TDA202 study

Even though multiple whole-cell pertussis (wP) vaccines of variable efficacy are in use, a stand-alone acellular pertussis (aP) booster vaccine was recently developed and licensed for vaccination in adolescents and adults in Thailand based on the results of the TDA 202 study done at the vaccine trial centre, faculty of tropical medicine, Mahidol University, Bangkok. Between July 2015-November 2016, a phase II/III randomized controlled study of two acellular pertussis vaccines manufactured by BioNet-Asia Co., Ltd.(aP standalone - Pertagen and TdaP combined vaccine, Boostagen) and a chemically-detoxified Tdap vaccine Adacel (manufactured by Sanofi Pasteur, Ltd) was conducted in Bangkok, Thailand. A total of 450 healthy subjects aged 12-17 years were enrolled into the study. During the study, the subjects were randomized to receive intramuscularly a booster dose (0.5 mL) of one of the 3 study vaccines. Both novel vaccines were similar to Adacel in terms of safety. Based on these results, both novel vaccines Pertagen and Boostagen, are now licensed and marketed in Thailand.[18,19]

Pertagen

Pertagen™ is BioNet Asia's acellular pertussis vaccine also called Viaskin® rPT; Viaskin®-PT. This is the only monovalent recombinant pertussis vaccine in the world, approved in a few countries of Asia for booster doses in adolescents and adults. It is prepared with recombinant

Table II. Catch up vaccination for unimmunized / partially immunized child

Catch up vaccination for unimmunized child	Catch up vaccination (1 to 7)years	Catch up vaccination (> 7-18 years)
Primary immunization	3 doses of DTwP / DTaP at 0, 4 weeks and 6 months interval	Tdap (preferred) at 0, and Td at 4 weeks and 6 months interval[#]
First Booster	After 6 months to 1 year	Td after 6 months to 1 year
Second Booster	Not necessary if received first booster after 4 years of age	Not necessary if received first booster after 4 years of age
Catch up vaccination for partially immunized child	Complete the remaining doses with DTP upto 7 years	Complete the remaining doses with Tdap after 7 years of age.

[#] Children receiving Tdap between 7-10 years of age do not require Tdap at 10-11 years of age.

Note: After the age of 18 years, only a single dose of Tdap/Td is recommended. Td vaccine can be used when indicated for prophylaxis of tetanus following injury or during pregnancy.

DNA technology and contains genetically-inactivated pertussis toxin (PTgen) and filamentous haemagglutinin (FHA) expressed by a unique genetically-engineered Bordetella pertussis strain. It produces more native and active pertussis toxoid than the ones included in currently available chemically detoxified acellular pertussis vaccines. PTgen both in stand-alone aP vaccine and in TdaP combinations was demonstrated to have a similar reactogenicity and safety profile as compared to currently available pertussis combination vaccines. This vaccine contains pertussis toxoid 5µg, FHA 5µg, aluminium hydroxide 0.3mg/dose, sodium chloride 4.38 mg for injection sufficient to make a volume of 0.5ml. It is injected as a single 0.5ml dose intramuscularly in the non-dominant deltoid.[18,19]

Boostagen

Boostagen (TdaPBioNet) was another vaccine produced with a recombinant B pertussis strain that was genetically inactivated by the introduction of mutations (Arg9Lys and Glu129Gly) in the ptx operon of the S1 gene. Each 0.5 mL dose of Boostagen contains 5µg pertussis toxin, 5µg of filamentous hemagglutinin and additionally, 7.5 Lf tetanus toxoid and 2.0 Lf diphtheria toxoid. The study vaccine was provided in a single-dose prefilled syringe and recommended for active immunization of individuals 11 years onwards. Safety of Boostagen in pregnant women and lactating mothers has not been assessed till date. More studies are needed for recommendation in these two particular groups.[18,19]

Immunogenicity of new vaccines

ELISA anti-PT, ELISA anti-FHA and anti-PT neutralizing antibody titers, as well as seroconversion rates ($\geq$ 4 fold increase) were significantly higher in adolescents and adults receiving PTgen-containing vaccine. The seroconversion rate defined as the proportion of subjects who reached at least a fourfold increase in titers of PT and FHA antibodies over baseline, was far superior at both 28 days and 1 year in subjects who received Pertagen or Boostagen vaccines as compared with those who received Adacel acellular pertussis vaccine. The fast-waning immunity of conventional acellular pertussis vaccines was amply illustrated by the difference in falloff of PT-neutralizing antibody over time. The PT-neutralizing antibody titers at 1 month and 1 year were 278 IU/mL and 77 IU/mL in the Pertagen group (containing PT-gen and FHA), 216 IU/mL and 67 IU/mL in the Boostagen group (produced with a recombinant B pertussis strain) and a mere 36 IU/mL and 12 IU/mL respectively for Adacel acellular pertussis vaccine.[17,18]

The Geneva University Hospitals in Switzerland is conducting the PertADO Geneva Trial,[20] the first clinical study in Europe evaluating Pertagen.™vaccine. The study aims to compare the safety and immunogenicity induced by booster vaccination with acellular pertussis vaccines including chemically or genetically detoxified Pertussis Toxin (PT) in adolescents previously immunized with acellular pertussis vaccines. Among 60 participants, half received one dose of recombinant aP vaccine plus a tetanus diphtheria booster vaccine and the other half a comparator licensed Tdap vaccine containing a chemically detoxified PT. No safety issues have been reported in any of the study participants during the followup period.

Pertaprime is another recently planned investigator driven randomized control trial to demonstrate non-inferior immunogenicity of vaccine containing genetically-inactivated pertussis toxin and FHA in comparison to vaccine with acellular component in healthy young Australian adults aged 18 to 25 years.[21]

Combination vaccines containing DPT

Immunogenicity and safety of a newly developed liquid hexavalent vaccine (DTwP-Hib/HepB-IPV-EasySix) was evaluated and compared with other commercially licensed Pentavac SD (DTwP-HepB/Hib) plus Imovax (IPV) vaccines in an open-label, randomized multi-centric trial in India.[22] The seroresponse rate and mean antibody titre for all vaccine components were comparable between EasySix and Pentavac SD-Imovax Polio combination. The vaccines had similar reactogenicity profiles and were well tolerated. The hexavalent vaccine was also co-administered with meningococcal vaccine and showed unaltered immunogenicity and efficacy without increase in side effects.[23]

Hexavalent vaccine was further tested for immunogenicity in an RCT (randomized controlled trial) in Spain which evaluated it in a mixed schedule including hexavalent / pentavalent / hexavalent schedule at 2, 4 and 6 months of age. It was found that mixed schedule evoked a satisfactory immune response for all the antigenic components one month after third dose of vaccine.[24] For routine immunization of infants, these combination vaccines are licensed to be used in a 3-dose vaccination series starting at the earliest from 6 weeks of age with a minimum interval of 4 weeks between doses, then a booster dose at age 15-18 months.

Adolescent and adult vaccination recommendations

It is a proven fact that immunity against pertussis wanes 10-12 years after completion of the primary immunization and boosters. A single dose of Tdap should

be used as booster in adolescents and adults if they have not received Tdap earlier. There is currently no recommendation for repeat Tdap administration. Td booster can be given every 10 years to those who have already received one dose of Tdap. According to a recent meta-analysis except for significant difference in gastrointestinal side effects like nausea and vomiting, acellular pertussis component vaccines are safe and have short-term effectiveness for adolescents and adults. The adverse event of acellular pertussis component was similar to or safer than that of placebo or other vaccines without pertussis antigen.[25]

Recommendations in pregnant women

Previously unimmunized pregnant women should receive one dose of Tdap followed by one dose of Td in a gap of four weeks for protection of the fetus. One dose should be administered at first contact with the health facility and second should be given at least 2 weeks before delivery for optimum effect. Single dose of Td or Tdap should be given in each subsequent pregnancy. Those who were fully immunized in childhood along with booster dose of Tdap in adolescence should only receive one dose of Tdap in pregnancy. In India since 2011 ACVIP has recommended use of Tdap to all pregnant women after 20 weeks of gestation.[26] A recent RCT published in 2019 assessed maternal pertussis vaccination and its effects on the immune response of infants aged up to 12 months in the Netherlands.[27] The study showed high pertussis toxin antibody concentrations at the age of 3 months and concluded that maternal vaccination supports a delay of the first pertussis vaccination in infants until at least age of 3 months as maternal antibody interference affects antibody concentrations after primary and booster vaccinations. Similar study done in Belgium showed lower level of anti pertussis toxin (PT) antibody even after one month of the fourth dose of DPT containing vaccine at 15 months of age in the group where mothers received aP containing vaccine Boostrix.[28] This aspect needs to be further evaluated for change of current vaccination schedule if needed.

Points to Remember

- ***DPT is an essential part of the immunization program in most countries and can be given alone or as a combination vaccine.***
- ***Paracetamol given as treatment or prophylaxis for fever does not impair the immunological response to DPT containing vaccines.***
- ***For unimmunized children aged of 1-7 years, the recommended catch up primary schedule is 3 doses with a minimum interval of 4 weeks between the first and the second dose and 6 months between the second and third doses.***
- ***A single dose of Tdap should be used as booster in adolescents and adults if they have not received Tdap earlier; during pregnancy one dose of Tdap should be administered at the first contact and second Td should be given at least 2 weeks before the delivery.***
- ***Absolute contraindications to all DPT containing vaccines are history of anaphylaxis or encephalopathy not attributable to any underlying cause and onset within 7 days of vaccination; progressive neurological disease is a relative contraindication for first dose of DTwP.***

References

1. Immunization, Vaccines and Biologicals [Internet]. Who.int. 2020 Available from: https://www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/passive/big_dtp3_global_coverage. Accessed on 30 September, 2020.
2. Worls Health Organisation. WHO vaccine-preventable diseases: monitoring system. 2020 global summary [Internet]. Apps.who.int. 2020 Available from: https://apps.who.int/immunization_monitoring/globalsummary. Accessed on 26 September, 2020.
3. World Health Organization. Pertussis [Internet]. 2020. Available from: https://www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/passive/pertussis/en/. Accessed on 26 September, 2020.
4. JeffersonT, Rudin M, Dipietrantonj C. Systemic review of the effects of pertussis vaccines in children. Vaccine 2003; 21:2003-2014.
5. WHO SAGE pertussis working group. Background paper. SAGE April 2014. Available from http://www.who.int/immunization/sage/meetings/2014/april/1_Pertussis_background_FINAL4_web.pdf?ua=; Last accessed on 26 September, 2020.
6. World Health Organization. Pertussis vaccines position paper [Internet]. 2020 Available from:https://www.who.int/immunization/policy/position_papers/pertussis/en/ Accessed on 17 September, 2020.
7. Warfel JM, Merkel TJ. Reply to Domenech de Cellès et al.: Infection and transmission of pertussis in the baboon model. Proc Natl Acad Sci USA. 2014 Feb 18;111(7): E718.
8. Balasubramanian S, Shah A, Pemde H, Chatterjee P, Shivananda S, Guduru V, et al. Indian Academy of Pediatrics (IAP) Advisory Committee on Vaccines and Immunization Practices (ACVIP) Recommended Immunization Schedule (2018-19) and Update on Immunization for Children Aged 0 Through 18 Years. Indian Pediatr 2018; 55(12):1066-1074.
9. National Health Mission. Strengthening Td10 and Td16 Vaccine Implementation Operational Guidelines and

Strategic Plan 2019 [Internet]. 2020 . Available from: https://nhm.gov.in/New_Updates_2018/NHM_ Components/Immunization/Guildelines_for_ immunization/Td_Report.pdf. Accessed on 26 September, 2020.

10. Langley JM, Predy G, Guasparini R, Law B, Diaz-Mitoma F, Whitstitt P, et al. An adolescent-adult formulation tetanus and diptheria toxoids adsorbed combined with acellular pertussis vaccine has comparable immunogenicity but less reactogenicity in children 4-6 years of age than a pediatric formulation acellular pertussis vaccine and diphtheria and tetanus toxoids adsorbed combined with inactivated poliomyelitis vaccine. Vaccine 2007; 25(6):1121-1125.
11. Gabutti G, Trucchi C, Conversano M, Zivelonghi G, Zoppi G, "Booster Vaccination: The Role of Reduced Antigen Content Vaccines as a Preschool Booster", BioMed Research International, vol. 2014, Article ID 541319, 10 pages, 2014. https://doi.org/10.1155/2014/541319.
12. World Health Organization. Information sheet. Diphtheria, Pertussis, Tetanus Vaccines. Available http://www.who.int/vaccine_safety/initiative/tools/DTP_vaccine_rates_information_sheet.pdf?ua=1. Accessed on 26 September, 2020.
13. Simonsen O, Kjeldsen K, Vendborg HA, Heron I. Revaccination of adults against diphtheria. I: Responses and reactions to different doses of diphtheria toxoid in 30-70-year-old persons with low serum antitoxin levels. Acta Pathol Microbiol Immunol Scand C. 1986 Oct; 94(5):213-218. doi: 10.1111/j.1699-0463.1986. tb02114.x. PMID: 3565027.
14. World Health Organization. Diphtheria position paper [Internet]. 2020. Available from: https://www.who.int/immunization/policy/position_papers/diphtheria/en/. Accessed on 17 September, 2020.
15. Sil A, Ravi M, Patnaik B, Dhingra M, Dupuy M, Gandhi D, et al. Effect of prophylactic or therapeutic administration of paracetamol on immune response to DTwP-HepB-Hib combination vaccine in Indian infants. Vaccine 2017; 35(22):2999-3006.
16. National Health Mission. Guidelines Immunization 2020. Available from: https://nhm.gov.in/New_Updates_2018/NHM_Components/Immunization/ Guildelines_for_immunization/Paracetamol_Guidelines.pdf. Accessed on 25 September, 2020.
17. World Health Organization. Recommendations to assure the quality, safety and efficacy of DT-based combined vaccines. WHO Technical Report Series No. 980, Annex 6. 2014.335–406. Available at http://who.int/biologicals/vaccines/Combined_Vaccines_TRS_980_Annex_6. Accessed on 25 September, 2020.
18. Clinical trials. 2-year Follow-up After a Single Dose Acellular Pertussis Vaccination - Full Text View - ClinicalTrials.gov [Internet]. 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT04113655. Accessed on 17 September, 2020.
19. Clinical trails. 3-year Follow-up After a Single Dose Acellular Pertussis Vaccination - Full Text View - ClinicalTrials.gov [Internet]. 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT04102137 Accessed on 17 September, 2020.
20. Clinical trials. The PertADO Geneva Trial - Full Text View - ClinicalTrials.gov [Internet]. 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT02946190. Accessed on 17 September, 2020.
21. Australia New Zealand Clinical Trials Registry (ANZCTR) Pertaprime: A study to assess the immune response and safety of a new pertussis vaccine (Pertagen®) in healthy young adults. [Internet]. Anzctr.org.au. 2020. Available from: http://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=377759. Accessed on 17 September, 2020.
22. Mohanty L, Sharma S, Behera B, Panwar S, Paliwal C, Gupta A et al. A randomized, open label trial to evaluate and compare the immunogenicity and safety of a novel liquid hexavalent DTwP-Hib/Hep B-IPV (EasySix™) to licensed combination vaccines in healthy infants. Vaccine 2018; 36(17):2378-2384.
23. Vesikari T, Borrow R, Da Costa X, Thomas S, Eymin C, Boisnard F et al. Concomitant administration of a fully liquid ready-to-use DTaP-IPV-HB-PRP-T hexavalent vaccine with a meningococcal ACWY conjugate vaccine in toddlers. Vaccine 2018; 36(52):8019-8027.
24. Martinón-Torres F, Boisnard F, Thomas S, Sadorge C, Borrow R. Immunogenicity and safety of a new hexavalent vaccine (DTaP5-IPV-HB-Hib) administered in a mixed primary series schedule with a pentavalent vaccine (DTaP5-IPV-Hib). Vaccine 2017; 35(30):3764-3772.
25. Xu J, Liu S, Liu Q, Rong R, Tang W, Wang Q et al. The effectiveness and safety of pertussis booster vaccination for adolescents and adults: A systematic review and meta-analysis. Medicine (Baltimore). 2019 Apr; 98 (16):e15281.
26. CDC. Updated recommendation for use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine (Tdap) in pregnant women and persons who have or anticipate having close contact with an infant aged <12 months-Advisory committee on immunization practices (ACIP), 2011. Morb Mortal weekly Resp 2011; 60:1424-1426.
27. Barug D, Pronk I, van Houten M, Versteegh F, Knol M, van de Kassteele J et al. Maternal pertussis vaccination and its effects on the immune response of infants aged up to 12 months in the Netherlands: an open-label, parallel, randomised controlled trial. Lancet Infect Dis 2019; 19(4):392-401.
28. Maertens K, Caboré R, Huygen K, Vermeiren S, Hens N, Van Damme P, et al.Pertussis vaccination during pregnancy in Belgium: Follow-up of infants until 1 month after the fourth infant pertussis vaccination at 15 months of age. Vaccine 2016; 34(31):3613-3619.

VACCINOLOGY I

POLIO VACCINES

***Abhay K Shah**
****Aashay A Shah**

Abstract: *Global Polio Eradication and Endgame Strategic Plan 2013-18 has emphasized complete and ultimate withdrawal of oral polio vaccines from all immunization programs across the globe. The term 'eradication' addresses wild polio virus and 'endgame' addresses vaccine associated paralytic polio and Vaccine Derived Polio Virus. The most crucial step in this direction was global implementation of synchronized withdrawal of type 2 Oral Polio Vaccine in 2016 through a switch from trivalent Oral Polio Vaccine to bivalent Oral Polio Vaccine. Still this can be associated with small but real risk of Vaccine Derived Polio Virus outbreaks. To address this vital issue, all Oral Polio Vaccine doses should ideally be replaced by inactivated poliovirus vaccine. Inactivated poliovirus vaccine introduction (in previously Oral Polio Vaccine only using countries) has increased global inactivated poliovirus vaccine demand, resulting in demand greater than supply. Such shortage has resulted in giving fractional doses of inactivated poliovirus vaccine intradermally as a risk mitigation in our national immunization program. Currently, Advisory Committee on Vaccines and Immunization Practice recommends bivalent oral polio vaccine at birth followed by inactivated poliovirus vaccine at 6 - 10 - 14 weeks stand alone or as part of Diphtheria Tetanus and whole cell pertussis vaccine / Diphtheria Tetanus and acellular pertussis vaccine combos and a booster of inactivated poliovirus vaccine / combo at 15-18 months and second booster at 4 to 6 years of age. An alternate schedule is two doses of intramuscular inactivated poliovirus vaccine instead of three for primary series if started at 8 weeks, with an interval of 8 weeks between two doses. All inactivated poliovirus vaccine immunized children should receive Oral Polio Vaccine on all supplementary immunisation activity days till 5 years of age. In case injectable inactivated poliovirus vaccine is not available or feasible child should be given 3 doses of bivalent oral polio vaccine with two fractional doses of Inactivated poliovirus vaccine (IPV) at a Government facility at 6 and 14 weeks or at least one dose of intramuscular inactivated poliovirus vaccine, either standalone or as a combination vaccine, at 14 weeks of age.*

Keywords: *Polio vaccines, VAPP, cVDPV, Polio eradication.*

Poliomyelitis (polio) is a highly infectious viral disease caused by any of the three poliovirus serotypes (types 1, 2 or 3) that largely affects children under 5 years of age. It is a paralytic disease resulting from involvement of motor neurons in the gray matter of the spinal cord, rarely brain and meninges. It is transmitted by person-to-person spread mainly through the faecal-oral route or, less frequently, by contaminated water or food. Virus multiplies in the intestine, from where it can invade the nervous system and cause paralysis.

Pathogen

Polioviruses belong to human enteroviruses of the Picornaviridae family and they share most of their biochemical and biophysical properties with other enteroviruses. They are non-enveloped viruses with a single-stranded RNA genome and a protein capsid. The 3 serotypes of polioviruses have different antigenic sites in the capsid proteins. They are resistant to inactivation by many common detergents and disinfectants, including soaps, but are rapidly inactivated by exposure to ultraviolet light. Virus is stable for months at +4°C and for several days at +30°C.

Disease

The incubation period is commonly 7-10 days (range 4-35 days). Viral replication occurs in nasopharynx. It presents in two forms- non-paralytic and paralytic forms. Non-paralytic form usually presents as features of aseptic meningitis with presence of fever and neck stiffness, severe headache and pain in limbs, back and neck. This form of polio lasts between 2 and 10 days and in almost all cases recovery is complete. Paralytic poliomyelitis occurs when

* Senior Pediatrician and Infectious Diseases Consultant
email: drabhaykshah@yahoo.com

** Fellow ISPAGHAN,
Associate Pediatrician,
Children Hospital, Ahmedabad.

poliovirus enters the central nervous system by peripheral or cranial nerve axonal flow and replicates in anterior horn cells (motor neurons) of the spinal cord. It causes acute flaccid paralysis (AFP) affecting the limbs, principally the legs, usually asymmetrically, while sensation remains intact. Persistent paralysis and permanent deformities are common sequelae. Mortality is predominantly associated with bulbar involvement. There is no specific anti-viral drug available for poliomyelitis. Neuromuscular sequelae are mitigated by physiotherapy and orthopaedic treatment. Fortunately, it is a vaccine preventable disease.

Global scenario

Wild poliovirus cases have decreased by over 99% since 1988, from an estimated 350 000 cases in more than 125 endemic countries then to 175 reported cases in 2019. Of the three types, wild poliovirus type 2 (WPV2) was eradicated in 1999 and certified in 2012 by the Global Commission for the Certification of the Eradication of Poliomyelitis.[1] No wild poliovirus type 3 (WPV3) has been detected anywhere in the world for over three years, since November 2012.[2] In a historic announcement on World Polio Day (24 Oct 2019), an independent commission of experts concluded that wild poliovirus type 3 (WPV3) has been eradicated worldwide. Following the eradication of smallpox and wild poliovirus type 2, this news represents a historic achievement for humanity. (https://www.who.int/news-room/feature-stories/detail/two-out-of-three-wild-poliovirus-strains-eradicated. Accessed on 29 Oct 2020.)[3] Five of the six WHO regions - representing over 90% of the world's population - are now free of the wild poliovirus, moving the world closer to achieving global polio eradication. Currently at the time of writing this paper, WPV1 cases reported were 61 and 142 in Afghanistan and Pakistan respectively. There were 19 cases of circulating vaccine-derived poliovirus type 1 (cVDPV1) and 603 cases of cVDPV2.

Indian Scenario

In 2009, India contributed to 60% of polio cases in the world. The last reported case of wild polio in India was in West Bengal on 13 January 2011. On 27 March 2014, the World Health Organization (WHO) declared India a polio free country. In spite of formidable barriers, the elimination of wild polioviruses (WPVs) types 3 and 1 in India, in 2010 and 2011, respectively was a major milestone in the global journey for polio eradication. These successes were achieved using oral poliovirus vaccines (OPV) containing live attenuated polioviruses - trivalent (tOPV with types 1, 2 and 3) monovalent polio 1, monovalent 3 and bivalent vaccine polio 1 and 3.

Vaccines

Historical aspects

Jonas Salk developed the first-ever licensed trivalent inactivated poliovirus vaccine (IPV), called Salk vaccine or IPV in 1955. IPV was introduced systematically in the US and was a huge success with 99% reduction in polio incidence when 3rd dose vaccine coverage was only <70% in children less than 5 years of age. Despite the success of Salk vaccine, the efforts to develop other polio vaccines, particularly the live oral vaccine were continued by US scientists namely Cox, Koprowski and Sabin. Albert Sabin conducted massive trials and had administered his oral vaccine to around 15 million subjects by July 1960.[4] The Sabin vaccine was found to offer durable immunity, fast onset of action, ease of administration by oral rather than through injection and prospect of possible 'contact immunization' to unvaccinated individuals through passage of live attenuated viruses in the faeces. Thus OPV was licensed initially in the USA as monovalent (m-OPV) and in 1963 as mixture of types 1, 2 and 3 (trivalent, tOPV). Until 1963, both Salk IPV and Sabin OPV were used in the USA and by 1968, Salk IPV was no longer being administered in the USA and manufacturers had stopped producing it. The risk of vaccine associated paralytic poliomyelitis (VAPP) with Sabin OPV was for the first time suspected in 1962 in USA. In 1964, a study done there confirmed a definite albeit a very small risk of VAPP associated with the use of Sabin vaccine.[5] By the end of 60s and early 70s, Sabin OPV was the main vaccine against poliomyelitis in majority of the countries world over.[4,5] Later, in 1974 when World Health Organization (WHO) launched the Expanded Program on Immunization (EPI), OPV was recommended for use in all low and middle income (LMI) countries. In 1988 when the Global polio eradication initiative (GPEI) was launched, the OPV was chosen as an exclusive tool for use in all these countries.[6,7] OPV was the only polio vaccine in US and in many European countries for almost next 35 years till late 1990s when the only polio cases occurring there were caused by the mutated-Sabin virus (VAPP).[4] In January 2000, the US switched back to exclusive use of IPV in place of OPV to thwart any possibility of polio due to VAPP.[5]

Although Sabin vaccine had clear-cut advantages over Salk IPV, few European countries like the Netherlands and Scandinavia continued exclusive use of the latter in their immunization programs.[5] Later in 1978, the improved, high-potency IPV was field tested in Mali and Burkino Faso by a research establishment formed by Salk, Cohen and Charles Mérieux. The new IPV, 'enhanced-potency

IPV' (eIPV) was found highly efficacious with just two doses.[8] Today only the improved eIPV is manufactured and supplied to whole world including USA.[9]

The vaccines

1. Inactivated / Injectable poliovirus vaccine (IPV) - IPV is given by injection and is available only in trivalent form containing the 3 virus serotypes PV1, PV2 and PV3 in the inactivated form.
2. Oral Polio vaccine (OPV) - given orally as drops and available as bivalent (PV1 and PV3) and monovalent OPV (PV1) as live attenuated form. Trivalent OPV is no longer available now.

Oral poliovirus vaccines (OPV): OPV is composed of live attenuated polioviruses derived of their parent WPV strains by passage in nonhuman cells to obtain the 3 vaccine strains (Sabin 1, 2 and 3). Attenuation of the virus greatly reduces its neurovirulence and transmissibility.[10]

There are several licensed formulations of OPV

(i) Monovalent OPVs against type 1 (mOPV1), type 2 (mOPV2) or type 3 (mOPV3)

(ii) Bivalent OPV (bOPV) containing types1 and 3

(iii) Trivalent (tOPV) containing types 1, 2 and 3- Discontinued since 2016.

Bivalent OPV (bOPV containing types 1 and 3 Sabin viruses) has been licensed and used in some settings since December 2009. Following the planned global switch from tOPV to bOPV in April 2016, tOPV is no longer available and has been replaced by bOPV. The only OPV containing serotype 2 will be type 2 monovalent OPV (mOPV2) and has been stockpiled only for emergency use.

Administration: OPV is administered as 2 drops (~0.1 mL), directly into the mouth.

Schedule: The schedule of OPV recommended in 'high income' countries was three doses given at intervals of four or more weeks, beginning at or after 2 months of age. The WHO EPI recommends a dose soon after birth and three more at 6, 10 and 14 weeks of age particularly for low and middle income (LMICs) countries. It is also used in supplementary immunization activity.

Storage: It is highly heat-sensitive and must be kept frozen for long-term storage or, after thawing, at temperatures between +2 °C and +8 °C for a maximum of 6 months. Vaccine vial monitors (VVM) give a visual indication of whether the vaccine has been kept at the correct temperature conditions.

Safety of OPV: OPV is extremely safe and effective. However, in some unforeseen situation it rarely causes Vaccine-associated paralytic polio (VAPP) and introduces Vaccine-derived polioviruses (VDPVs) in the community. All available evidence indicates that OPV is non-teratogenic and safe to administer to pregnant women and HIV-infected persons.

Vaccine-associated paralytic polio (VAPP): Sabin genotypes of live attenuated polioviruses can regain neurovirulence and very rarely (at a rate of approximately 2 to 4 events per 1 million birth cohorts per year in OPV using countries)[11] can cause VAPP either in the vaccinated child ('recipient VAPP', occurring within 4-40 days of receiving OPV) or in a close contact ('contact VAPP').[9] 26%-31% of VAPP cases are caused by Sabin type 2 viruses.[11] Hence, safety profile of bOPV is assumed to be better than that of tOPV. In the industrialized countries VAPP occurs mainly in early infancy associated with the first dose of OPV and decreases sharply (>10 fold) with subsequent OPV doses. In lower-income countries which experience relatively lower rates of vaccine seroconversion, VAPP may occur with second or subsequent doses of OPV, common among children aged 1-4 years and the decline with subsequent doses is more gradual.[12,13] These differences are because of lower immune responsiveness to OPV and higher prevalence of maternally derived antibody in populations in low-income settings. As the virus causing VAPP is not transmissible, there are no outbreaks associated with VAPP. Thus, VAPP is a risk to an individual and not to the community.

Vaccine-derived polioviruses (VDPVs): The attenuated viruses in live OPV vaccines (Sabin viruses) during their prolonged replication in an individual or in a community, sometimes re-acquire the neurovirulence and transmissibility characteristics of WPV. So, in areas where OPV coverage is sub-optimal, Sabin genotypes can spread among non-immune children, leading to rare lineages of vaccine-derived polioviruses (VDPVs) that have regained both the neurovirulence and transmissibility characteristics of WPV. When it is associated with sustained person-to-person transmission and is circulating in the environment it is labelled as circulating VDPV (cVDPV). "Persistent cVDPVs" refer to cVDPVs known to have circulated for more than six months. Circulating VDPVs can cause polio outbreaks when routine immunization or supplementary immunization activities are poorly conducted and a significant proportion of the population is left susceptible to poliovirus. Hence persistent cVDPVs represent programmatic failures to contain the cVDPV outbreak within 6 months of detection. Low vaccination coverage

is a major risk factor for cVDPV outbreaks, as seen in Nigeria and Pakistan, and cVDPVs can also be imported and spread in any under-vaccinated community in a developed country.[14]

There are 3 types of VDPV:

1) Circulating vaccine derived polio virus (cVDPV): As described above
2) Immunodeficiency related vaccine derived polio virus (iVDPV) reported in immunodeficient patients like primary B-cell or combined immunodeficiency disorders. They have prolonged infections after exposure to VDPV infections, due to prolonged viral shedding.
3) aVDPV (ambiguous vaccine derived polio virus) currently have unclassified source (i.e., a single isolate from a healthy or non-immunodeficient person; environmental isolates without an associated AFP case).

Immunogenicity and effectiveness of OPV: OPV when administered behaves like a natural infection to wild polio virus (WPV) and it immunizes after infection ('take') in the intestine. It generates both humoral and mucosal immunity.[15] The 'take' frequency and antibody response rate are lower in many LMI countries than in high income countries. The reduced antibody response to OPV in children in some low-income settings is probably attributed to complex factors like high levels of maternal antibody, poor intestinal immunity in malnourished children, diarrhea at the time of vaccination, household exposure to other OPV recipients, the vaccine and its delivery and the prevalence of other enteric infectious agents.[16] Nevertheless OPV induces high levels of intestinal immunity and prevents wild virus shedding (and therefore transmission) resulting in population protection.

In high-income countries, seroconversion rates in children following administration of 3 doses of tOPV approaches 100% for all 3 poliovirus types.[17,18] However, in some developing countries, the same 3-dose course of tOPV in children was found to induce detectable antibodies in only 73% (range, 36%-99%), 90% (range 77%-100%) and 70% (range, 40%-99%) to poliovirus type 1, 2 and 3, respectively.[19] Type 2 is dominant in tOPV and interferes with 'take' and immune response to types 1 and 3. Hence, additional doses of OPV are necessary to close immunity gaps particularly to types 1 and 3. As per one clinical trial in India, cumulative 2-dose seroconversion for poliovirus type 1 was 90% for mOPV1 and 86% for bOPV compared to 63% for tOPV; for type 3, 84% for mOPV3 and 74% for bOPV compared to 52% for tOPV.[20]

Birth dose of OPV-Why?: A dose of OPV administered at birth, or as soon as possible after birth, significantly improves the seroconversion rates to the subsequent doses. Birth dose or the first OPV dose given at a time when the infant is still protected by maternally-derived antibodies may also prevent VAPP. Seroconversion to birth doses varies from region to region e.g. low rates in India (10%-15%), median rates in Egypt (32%) and high rates in South Africa (76%). However, in general, the birth dose increases the levels of poliovirus neutralizing antibodies and seroconversion rates achieved after completion of the routine vaccination schedule.[21,22]

Duration of protection with OPV: Protective immunity induced by active immunity either by vaccination or exposure to poliovirus is usually lifelong against paralytic polio for an individual. It is measured by circulating antibody titre. IgM antibody which becomes detectable as early as 2-3 days after infection, usually disappears after 2-3 months, while IgG becomes the predominant antibody and may last for life.[15] Sometimes, antibody titres decline over time and may fall below detectable levels, yet in immunocompetent individuals it is not a risk for paralytic disease. Hence, seroprevalence may not reflect the true immune status of a given population. The intestinal immunity induced by OPV does not persist beyond a year. Therefore sustained high seroprevalence is very important to curtail community transmission.

Inactivated Poliovirus Vaccine (IPV)

IPV is made by formalin inactivation of laboratory-maintained and vero-cell grown wild poliovirus (WPV) strains known as Mahoney (type 1), MEF-1 (type 2) and Saukett (type 3). In addition to formaldehyde, IPV contains traces of streptomycin, neomycin or polymyxin B. Some formulations of IPV contain 2-phenoxyethanol (0.5%) as a preservative for multi-dose vials. IPV formulations do not contain thiomersal, which is incompatible with IPV antigenicity, hence in combination products with DTP it is either avoided or replaced with 2-phenoxy-ethanol. All current IPV vaccines have substantially greater antigenicity and are termed 'enhanced potency IPV' (eIPV). IPV is available either as a stand-alone product or in combination with one or more other vaccine antigens including DTP, hepatitis B, or Hib.

Storage: The vaccine should be refrigerated to preserve potency but not frozen as this could diminish potency. Current 10-dose and 5-dose IPV vials can be used according to the WHO multi-dose vial policy and kept for up to 28 days after opening.

Route of administration: IPV can be administered by subcutaneous or intramuscular injection as per

manufacturer specifications. When combined with an adjuvanted vaccine the injection must be intramuscular. A fractional dose of stand-alone IPV can also be administered via the intradermal route.

Safety of IPV: IPV is considered very safe, whether given alone or in combination with other vaccines. Minor adverse events are transient and include local erythema (0.5%-1%), induration (3%-11%) and tenderness (14%-29%).[23,24] When IPV is administered along with DTP or as a combined DTP-IPV reactogenicity was similar to administration of DTP alone. Although IPV is considered safe, there is a risk of exposure to the wild type strain during the manufacturing process and requires very high biosafety - Biosafety level 4 (BSL4) measures.

Immunogenicity, efficacy and effectiveness of IPV: IPV has been highly effective in eliciting humoral antibody responses in both high income and low-income settings. The immunogenicity of IPV schedules depends on the age at administration and number of doses, (due to interference by maternal antibodies). Two or three doses, given at appropriate age intervals, especially after maternal antibody wanes and with intervals of over 8 weeks, are sufficient for 100% antibody response to all the three types of polioviruses, with quite high titers.[25,26]

Vaccination schedules vary between different countries: A single IPV dose generally seroconverts a proportion of vaccinees but induces immune memory (primes) in the majority of the remaining seronegative children.[15] One dose and preferably two doses are needed for 'priming' so that subsequent doses of it will provide a rapid and stronger immune response. A second dose given two or more months later completes priming and also acts as partial booster.[25] Long-lasting immunity will be achieved with a third dose (booster) given ideally several months later.[27] A fourth dose is also given a few years later in some countries (e.g. USA, UK, Sweden).[27] A study of immunogenicity of a 3-dose schedule in Puerto Rico found seroconversion rates of 85.8%, 86.2% and 96.9% for serotypes 1, 2 and 3 respectively on a 6, 10, 14 week schedule, compared with 99.6%, 100% and 99.1% on a 2, 4, 6 month schedule. Seroconversion was strongly dependent on the age at vaccination.[28,29]

Combined use of OPV+IPV

Combined IPV+OPV schedules appear to correct for the lower immunogenicity of OPV in developing countries.[29,30] IPV induces pharyngeal immunity similar to that of OPV, but much less intestinal immunity.[31] This limitation can be taken care by using both forms of polio vaccines in combination. Effective mucosal immunity to protect against WPV infection may be needed both at the pharyngeal and intestinal levels.

OPV before IPV: Immunogenicity of IPV in an OPV-exposed population has been found to be more efficacious. In OPV-immunized children, a dose of IPV boosts both humoral and intestinal immunity severalfold higher than a dose of OPV.[32,33] This is the basis for the endgame recommendation to give one dose of IPV at the time of the third dose of OPV, at age 14 weeks or more.[31] This was also proved in a multiarm trial in Moradabad, India which compared a supplemental dose of IPV at 6-9 months to children who had completed a primary series of tOPV plus multiple doses of mOPV, to boosting with a standard and higher potency type 1 mOPV.[15]

IPV before OPV: In developed countries where elimination of polio was achieved, VAPP was seen as a major public health problem. This issue was addressed by using IPV in a sequential IPV to OPV schedule. This schedule provides advantages of both IPV and OPV while minimizing adverse reactions. Initial immunization with IPV to promote humoral immunity, which gives children protection from VAPP and subsequent OPV vaccination induces higher levels of intestinal immunity and maintas population-level protection. The introduction of one dose of IPV prior to vaccination with OPV led to the elimination of VAPP in Hungary.[10]

Polio eradication and endgame - Role of OPV and IPV in polio eradication

In 2013, the World Health Organization (WHO) unveiled the 'Polio Eradication and Endgame Strategic Plan 2013-2018', in which the term 'eradication' addresses WPVs and 'endgame' addresses VAPP and VDPVs.[34] A polio-free world means complete interruption of transmission of WPVs as well as elimination of all polio disease including VAPP and VDPVs.[35]

Steps and rationale

The steps involved were

1. By the end of 2015, introduce at least 1 dose of IPV into all routine immunization systems, at least 6 months before the switch from trivalent oral polio vaccine (tOPV) to bivalent oral polio vaccine (bOPV, containing types 1 and 3 poliovirus).

2. During 2016, switch from tOPV to bOPV, which does not contain type 2 virus, in routine immunization and polio campaigns.

3. Plan for the eventual withdrawal of all OPV.

Because type 2 virus accounts for more than 95% of cVDPV outbreaks detected in recent years and approximately 30% of VAPP cases, it was most crucial to replace trivalent OPV (tOPV), which protects against types 1, 2 and 3, with a bivalent OPV (bOPV), which protects against types 1 and 3. Discontinuing OPV will prevent VAPP, but the resultant immunity gap in the community will constitute a risk for the emergence of cVDPVs and polio outbreaks. This risk can be mitigated by using IPV. Strategic Advisory Group of Experts on Immunization (SAGE) has recommended the introduction of at least one dose of IPV by the end of 2015 to mitigate the risks associated with OPV cessation. Therefore, only a single dose of IPV is incorporated into the immunization schedule, at 14 weeks of age with DTP3, or first contact after 14 weeks, to ensure good immunogenicity without interference from maternal antibody.

Importance of one dose of IPV

One dose induces seroconversion in 19-41% for type 1; 32- 63% for type 2 and 28-54% for type 3.[36] However, remaining children are immunologically primed so that a second dose results in a rapid anamnestic response. Thus, IPV will mitigate the risk of cVDPV2 emergence after the tOPV-bOPV switch and will also enhance immunity against types 1 and 3 in case of unprecedented virus re-introduction from sources like importation or laboratory leak. In case of an outbreak of cVDPV2, a dose of IPV will rapidly boost immunity in primed children.[36] For an outbreak response OPV has been the vaccine of choice for 'mop-up' vaccination program as it is easy to administer orally and is definitely a cheaper intervention. IPV-primed children will mount quicker and higher humoral antibody response to a dose of mOPV2 than in exclusively OPV-inoculated children and also avoids the risk of re-seeding vaccine virus type 2 while using mOPV2 given in campaigns.[37] The age cohorts before the switch would have already received OPV doses and can be rapidly boosted with both humoral and intestinal immunity, helping in interruption of transmission. Children born after the switch would either have received IPV or not received due to incomplete coverage - both groups will benefit from a dose of IPV.[31]

IPV availability issues and fractional dose of IPV

IPV introduction (in previously OPV-only using countries) has increased global IPV demand, stand-alone as well as that used in combination vaccines, from about 80 million doses in 2013 to about 200 million doses in 2016, resulting in demand greater than supply. Such shortage has resulted in giving fractional doses of IPV intradermally (ID). In fractional IPV (fIPV), only one-fifth (0.1 ml) of the full dose of intramuscular IPV (0.5 ml) is administered intradermally, which offers potential cost reduction and allows immunization of a larger number of persons with a given vaccine supply.[38] The results indicate that 2 fractional doses of IPV provide higher seroconversion rates than a single full dose, as shown in Cuba (63% when given at age 4 months) and in Bangladesh (39% when given at age 6 weeks).[39] The excellent immunogenicity of ID fractional (one-fifth) dose of IPV was also documented in pioneering research in the Cuban study. Based on these studies and in the light of global IPV shortage SAGE recommended to consider an IPV schedule of two fractional doses (0.1 ml each) at ages 6 and 14 weeks in lieu of one full dose (0.5 ml) at age 14 weeks. During this period all OPV using countries must continue giving bOPV as per the usual schedule under the Expanded Program on Immunization (EPI). Government of India has already started this regime of fIPV. Multi-dose IPV vial permits use of vials up to 28 days from the date of first use and this will minimize wastage particularly in immunization sessions, wherein the number of vaccine recipients is less. The endgame plan also envisages the opportunity to discontinue bOPV in the near future.[31]

The Polio Endgame Strategy 2019-2023[40]

The aim is to overcome the final hurdles to eradication and move toward sustaining a polio-free future. There are 3 important components of this plan and are described in Table I.

1. Eradication,
2. Integration,
3. Containment and Certification

WHO Recommendations[30]

- WHO recommends that all children worldwide should be fully vaccinated against polio and every country should seek to achieve and maintain high levels of coverage with polio vaccine in support of the global commitment to eradicate polio.
- WHO no longer recommends an OPV-only vaccination schedule. For all countries currently using OPV only, at least one dose of IPV should be added to the schedule.
- In polio-endemic countries and in countries at high risk for importation and subsequent spread, WHO recommends a bOPV birth dose (a zero dose) followed

Table I. Components of polio endgame strategy 2019-2023.

Polio Eradication & Endgame Strategic Plan 2013-2018

1. Detect and interrupt all poliovirus transmission.
2. Strengthen Immunization systems and withdraw oral polio vaccine.
3. Contain poliovirus and certify interruption of transmission.
4. Plan polio's legacy.

Continue	Improve	Innovate
Goal 1: Eradication		
Immunization campaigns Stockpile management AFP & environmental surveillance	Community engagement Accountability & supportive management Surge capacity Expand environmental surveillance network Communication for eradication	Regional hub for partnership support to endemic country teams Expanded age groups for SIAs Engagement of development & humanitarian actors for basic community needs Rapid response team for outbreaks Invest in antivirals & new IPV
Goal 2: Integration		
bOPV & IPV delivered as part of national Immunization schedules	Integration of polio surveillance with VPD surveillance Engagement with CSOs to better reach communities Joint delivery and/or enhanced coordination between polio & other VPDs SIAs	Joint accountability framework with GAVI & immunization partners for systematic collaboration Formalized MoU between WHO emergency programme & GPEI to harmonize outbreak & emergency response Immunization system recovery/ strengthening included in all outbreak response Harmonized data systems: POLIS & WISE
Goal 2: Certification & Containment		
Certification processes Poliovirus-essential facility certification process National containment surveys and inventories & inventories guidelines	Containment guidance Communications (including VDPV plans) Data quality metrics	Introduce genetically stable vaccine strains to eliminate the need to use and retain live poliovirus
Enabling Areas		
Increase female workers and leaders at all levels Promote staff rotations and incentive packages Establish focused support to polio transition activities		

Polio Post-Certification Strategy

Detect and respond

Promptly detect any poliovirus in a human or in the environment and rapidly respond to prevent transmission

Protect populations

Withdraw the oral live attenuated polio vaccine (OPV) from use and immunize populations with inactivated polio vaccine (IPV) against possible re-emergence of any poliovirus.

Contain poliovirus

Ensure potential sources of poliovirus are properly contained or removed.

Adopted from polio endgame strategy 2019-23 Eradication, Integration, Containment and Certification http://polioeradication.org/wp-content/uploads/ 2019/06/ english-polio-endgame-strategy.pdf.

by a primary series of 3 bOPV doses and at least one IPV dose.

- The primary series can be administered according to the regular schedules of national immunization programmes, for example at 6, 10, and 14 weeks (bOPV1, bOPV2, bOPV3+IPV), or at 2, 4 and 6 months (bOPV1, bOPV2+IPV, bOPV3 or bOPV1, bOPV2, bOPV3+IPV).
- Both OPV and IPV may be co-administered with other infant vaccines.
- For infants starting the routine immunization schedule late (age >3 months) the IPV dose should be administered at the first immunization contact along with bOPV and the other routinely recommended vaccines.
- In countries with high vaccination coverage (e.g. 90%-95%) and low importation risk (neighbouring countries and major population movement all having similarly high coverage) an IPV-bOPV sequential schedule with, the initial administration of 1 or 2 doses of IPV should be followed by ≥2 doses of bOPV to ensure both sufficient levels of protection in the intestinal mucosa and a decrease in the burden of VAPP.
- An IPV-only schedule may be considered in countries with both sustained high immunization coverage and the lowest risk of both WPV importation and transmission. A primary series of 3 doses of IPV should be administered beginning at 2 months of age. If the primary series begins earlier (e.g. with a 6, 10 and 14-week schedule) then a booster dose should be given after an interval of ≥6 months (for a 4-dose schedule).
- To mitigate the risk of undetected transmission, WHO recommends that endemic countries and countries with a high risk of WPV importation should not switch to an IPV-only or a sequential IPV - bOPV schedule at this time. The 3 bOPV+1 IPV schedule as currently recommended should be adopted and supplemental immunization activities should continue to support intensive efforts to eliminate poliovirus transmission.
- A sequential IPV-bOPV schedule or IPV-only schedule can be considered in order to minimize the risk of VAPP, but only after a thorough review of local epidemiology.
- Polio vaccine (IPV or bOPV) may be administered safely to asymptomatic HIV-infected infants. HIV testing is not a prerequisite for vaccination. bOPV is contraindicated in severely immunocompromised patients.
- Before travelling abroad, persons residing in countries with active transmission of a wild or vaccine-derived poliovirus should have completed a full course of polio vaccination in compliance with the national schedule, and received one dose of IPV or bOPV within 4 weeks to 12 months of travel, in order to boost intestinal mucosal immunity and reduce the risk of poliovirus shedding. Travellers to infected areas should be vaccinated according to their national schedules. All health-care workers worldwide should have completed a full course of primary vaccination against poliomyelitis.

ACVIP (2020-21) recommendations for polio vaccines

ACVIP recommends bOPV at birth followed by IPV at 6 - 10 - 14 weeks standalone or as part of DTwP/DTaP combos and a booster of IPV/combo at 15-18 months and second booster dose at 4-6 years of age.[41] In the IAP schedule, IPV is the main preventive measure against polio.

An alternate schedule is two doses of intramuscular IPV instead of three for primary series if started at 8 weeks, with an interval of 8 weeks between two doses is an alternative.

All IPV immunized children should receive OPV on all supplementary immunisation activity (SIA) days till 5 years of age.

ACVIP 2020-21 also reemphasizes the need of IPV in primary series. No child should be administered only pentavalent vaccine and bOPV in infancy without IPV (2 doses of fractional dose intradermal IPV at 6 weeks and 14 weeks or a single dose of full-dose intramuscular IPV/ hexavalent combination at 14 weeks).

If hexavalent vaccines are unaffordable/unavailable, the infant must be referred to a government healthcare facility for the primary immunization as per UIP schedule.

Rationale for IPV booster at 4-6 years of age

It needs to be emphasized that until worldwide polio eradication is achieved, cases of imported WPV from endemic neighboring countries or cases of cVDPV, remains a real threat unless population immunity is maintained by vaccinating children adequately in their early years of life. Outbreaks of cVDPVs have occurred in countries which have been polio free for several years.

IPV is immunogenic in an EPI schedule (6-10-14 weeks), but the titers achieved and the seroconversion rates may be lower, compared with vaccination of infants at older ages (2-4-6 months) as

followed in industrialized world.India is polio free for over 9 years, there have been no cVDPV outbreaks since past 4 years and no detection of polio virus in environmental samples over past 3 years. There is presently no evidence of polio transmission. If vaccination coverage goes down, immunity gaps may develop and there may be a need for polio vaccination at 4-6 years or older age groups. Persistence of antibodies only up to the school-entry age has been demonstrated, so all IPV using countries recommend a school age booster.[42,43]

In industrialized and developed world using only IPV 5 year booster is routinely recommended to take care of waning immunity and resultant immunity gap. Studies carried out in such countries have shown definite decline in protective antibodies after toddler booster and supports the need for school-entry booster with IPV.

NIP recommended schedules for polio vaccines.[44]

Birth dose and 3 doses of bOPV at 6, 10 and 14 weeks with a booster at 18 months of age. In addition, fIPV was introduced in 8 States and Union Territories of India in April 2016. By the end of 2016, India expanded fIPV nationwide and this policy is targeting now the entire 26 million annual birth cohort of India, with two fIPV doses at ages 6 and 14 weeks.

Novel approaches in polio vaccines

Globally, the entire world is moving away from OPV and since 2013, over 90 countries have introduced IPV in their EPI schedule.[45] Currently almost all IPV is made from laboratory maintained fully virulent WPVs, which demands an exceptionally extraordinary safety measures for fear of unprecedented breach. Some of the novel approaches have emerged and studied as an antigen sparing endeavour imparting safer approaches.

Monovalent IPV-2

A higher antigen content monovalent IPV type 2 (m-IPV2) could be an option for the polio end game immunization strategies as a source of more effective primary immunogenicity against type 2, particularly during the period when bOPV would be used in routine immunization (RI). A Phase I study on safety in adults with m-IPV2 is now completed, and a Phase II study on safety and immunogenicity in infants is underway with this product.[15]

Adjuvanted IPV

Formulations of IPV are combined with an adjuvant to improve the immune response and decrease the amount of polio antigens needed. Adjuvants have been shown to enhance onset, magnitude, duration and/or quality of the immune response translating into reduction in antigen dose or in the number of doses required, which could translate into the reduction of the costs of vaccination. IPV formulations adjuvanted with aluminum salts, have been shown to have dose-sparing effect and are already widely used in vaccines, including IPV containing combination vaccines.[15]

Sabin IPV

This is a novel future prospect to minimize the risk of reintroduction of WPV from IPV manufacturing units wherein attempts are made to develop IPVs that are formulated from an attenuated live poliovirus.[15] Successful development of IPV based on the attenuated Sabin virus strains has led to the licensure of the Sabin IPV in Japan and subsequent introduction of DTP-Sabin IPV formulations in routine immunization program in the country.[15]

nOPV

Novel OPV2 (nOPV) are candidate vaccines designed to stabilize the poliovirus genome and minimize the acquisition of neurovirulence, to provide safer alternatives for outbreak control of cVDPVs in the era following OPV2 cessation. 2 such vaccines are in phase 2 trials.

Points to Remember

- ***Poliomyelitis, a serious crippling disease is now on the verge of eradication. Role of both inactivated polio vaccine (IPV) and oral polio vaccine (OPV) is indispensable. Among these, OPV is the major contributor to India's success story in polio elimination and eradication.***
- ***OPV is extremely safe and effective, cheap and easy to administer. It imparts excellent gut immunity. In some unforeseen situations it rarely causes Vaccine-associated paralytic polio (VAPP) and Vaccine-derived polioviruses (VDPVs).***
- ***Global Polio Eradication and Endgame Strategic Plan 2013-18 has emphasized complete and ultimate withdrawal of oral polio vaccines (OPV) from all immunization programs across the globe.***
- ***All OPV doses should ideally be replaced by IPV. If not feasible child should continue 3 doses of bOPV with 2 doses of fIPV at public sector.***
- ***An IPV-only schedule may be considered in countries with both sustained high immunization coverage and***

the lowest risk of both WPV importation and transmission. A primary series of 3 doses of IPV should be administered beginning at 2 months of age. If the primary series begins earlier (e.g. with a 6, 10 and 14-week schedule) then a booster dose should be given after an interval of ≥6 months (for a 4-dose schedule).

- ***To mitigate the risk of undetected transmission, WHO recommends that endemic countries and countries with a high risk of WPV importation should not switch to an IPV-only or a sequential or 2 doses of fIPV - bOPV schedule at this time. The 3 bOPV+ 1 IPV or two doses of fIPV schedule as currently recommended should be adopted and supplemental immunization activities should continue to support intensive efforts to eliminate poliovirus transmission.***
- ***Combined IPV+OPV schedules appear to correct for the lower immunogenicity of OPV in developing countries. IPV induces pharyngeal immunity similar to that of OPV, but much less intestinal immunity.***
- ***Birth dose OPV and OPV in SIAs till 5 years of age are very important.***

References

1. Global Polio Eradication Initiative >News stories. Global eradication of wild poliovirus type 2 declared [Internet].Available from: http://www.polioeradication.org/mediaroom/newsstories/Global-eradication-of-wildpoliovirus-type-2-declared/tabid/526/news/1289/Default. aspx. Accessed September 24, 2020.
2. Global Polio Eradication Initiative > Data and monitoring > Polio this week >Wild poliovirus list Available from: http://www.polioeradication.org/Dataandmonitoring/Poliothisweek/Wildpolioviruslist.aspx. Accessed September 24, 2020.
3. Polio this week as of 23 September 2020 Available from http://polioeradication.org/polio-today/polio-now/this-week/ Accessed 28 September 2020.
4. Robbins FC. The History of Polio Vaccine Development. In: Plotkin SA, Orenstein WA, Ed. Vaccines, 4th Edn. Philadelphia: PA Saunders; 2004; pp17-30.
5. Blume S, Geesink I. A brief history of polio vaccines. Science. 2000; 288:1593-1594.
6. World Health Organization. The Expanded Programme on Immunization. Available from: http://www.who.int/immunization/programmes_systems/supply_chain/benefits_of_immunization/en/ Accessed on 24 September 2020.
7. World Health Assembly. Global eradication of poliomyelitis by the year 2000. Resolution 41.28. Fortyfirst World Health Assembly, Geneva, 2-13 May 1988. Available from : https://www.who.int/ihr/polio resolution 4128en.pdf?ua=1. Accessed on 24 september 2020
8. Van Wezel AL, Van Steenis G, Van der Marel P, Osterhaus AD. Inactivated poliovirus vaccine: current production methods and new developments. Rev Infect Dis 1984; 6(suppl 2):S335-340
9. Vashishtha VM, S Kamath. Inactivated poliovirus vaccine: current production methods and new developments Indian Pediatr 2016; 53(1):S65-69.
10. World Health Organisation. Polio vaccines: WHO position paper – March, 2016 Available from: https://www.who.int/wer/2016/wer9112.pdf?ua=1. Accessed on 24 September 2020.
11. Platt LR, Estívariz CF, Sutter RW. Vaccine-associated paralytic poliomyelitis: a review of the epidemiology and estimation of the global burden. J Infect Dis 2014; 210 suppl 1:S380-389.
12. Kohler KA, Banerjee K, Hlady WG, Andrus JK, Sutter RW. Vaccine-associated paralytic poliomyelitis in India during 1999: decreased risk despite massive use of oral polio vaccine. Bull World Health Organ 2002; 80(3):210-216.
13. Considerations for the timing of a single dose of IPV in the routine immunization schedule. Available from:http://www.who.int/immunization/sage/meetings/2013/november/1_Sutter_IPV_age_tech_background_14_October_2013_final.pdf, accessed February 2016.
14. Jenkins HE, Aylward RB, Gasasira A, Donnelly CA, Mwanza M, Corander J, et al. Implications of a circulating vaccine-derived poliovirus in Nigeria for polio eradication. N Eng J Med 2010; 362:2360-2369.
15. Bandyopadhyay AS, Garon J, Seib K, Orenstein WA. Polio vaccination: past, present and future. Future Microbiol 2015; 10:791-808.
16. Bar-On ES, Goldberg E, Hellmann S, Leibovici L. Combined DTP-HBV-HIB vaccine versus separately administered DTP-HBV and HIB vaccines for primary prevention of diphtheria, tetanus, pertussis, hepatitis B and Haemophilus influenzae B (HIB). Available from Cochrane Database Syst Rev. 2012 Apr 18; (4):CD005530. doi: 10.1002/14651858.CD005530.pub3. PMID: 22513932.
17. McBean AM, Thoms ML, Albrecht P, Cuthie JC, Bernier R. Serologic response to oral polio vaccine and enhanced potency inactivated polio vaccines. Am J Epidemiol 1988; 128:615-628.
18. Reporting and classification of vaccine derived polioviruses. Available at http://www.polioeradication.org/Portals/0/ Document/Resources/VDPV_Reporting Classification.pdf, accessed February 2016.
19. Patriarca PA. Factors affecting the immunogenicity of OPV in developing countries: a review. Rev Infect Dis 1991; 13(5):926-939.

20. Estívariz CF, Jafari H, Sutter RW, John TJ, Jain V, Agarwal A, et al. Immunogenicity of poliovirus vaccines administered at age 6-9 months in Moradabad District, India: A randomized controlled phase 3 trial. Lancet Infect Dis 2012; 12:128-135.
21. John TJ. Immunisation against polioviruses in developing countries. Rev Med Virol 1993; 3:149-160.
22. De-Xiang D, Hu Xi-min, Liu Wan-jun, Li Jin-shen, Jin Yu-cai, Tan Shun-ge, et al. Immunization of neonates with trivalent oral poliomyelitis vaccine (Sabin). Bull World Health Organ 1986; 64(6):853-860.
23. John TJ, Jain H, Ravishankar K, Amaresh A, Verma H, Deshpande J, et al. Monovalent type 1 oral poliovirus vaccine among infants in India: report of two randomized double-blind controlled clinical trials. Vaccine 2011; 29(34):5793-5801.
24. Vidor E. Poliovirus vaccine-inactivated. In: Plotkin SA, Offit PA, Orenstein WA Edward KM editors. Plotkin's vaccines. 7th Edn. Philadelphia: Elsevier; 2018; pp841-865.
25. Iqbal S, Shi J, Seib K, Lewis P, Moro PL, Woo EJ, et al. Preparation for global introduction of inactivated poliovirus vaccine: safety evidence from the US Vaccine Adverse Event Reporting System, 2000-12. Lancet Infect Dis 2015; 15(10):1175-1182.
26. Simoes EF, Padmini B, Steinhoff MC, Jadhav M, John TJ. Antibody response of infants to two doses of inactivated poliovirus vaccine of enhanced potency. Am J Dis Child 1985; 139:977-980.
27. Resik S, Tejeda A, Lago PM, Diaz M, Carmenates A, Sarmiento L, et al. Randomized controlled clinical trial of fractional doses of inactivated poliovirus vaccine administered intradermally by needle-free device in Cuba. J Infect Dis 2010; 201:1344-1352.
28. Carlsson RM, Claesson BA, Fagerlund E, Knutsson N, Lundin C. Antibody persistence in 5 year-old children who received a pentavalent vaccine in infancy. Pediatr Infect Dis J 2002; 21:535-541.
29. Dayan GH, Thorley M, Yamamura Y, Rodríguez N, McLaughlin S, Torres LM, et al. Serologic response to inactivated polio vaccine: a randomized clinical trial comparing 2 vaccination schedules in Puerto Rico. J Infect Dis 2007; 195:12-20.
30. World Health Organisation. WHO position paper on Polio vaccines March 2016, Available from https://www.who.int/immunization/policy/position_papers/polio/en/ Accessed on 30 September 2020.
31. Garon J, Orenstein W, John TJ. The Need and Potential of Inactivated Poliovirus Vaccine. Indian Pediatr 2016; 53 Suppl 1:S2-S6.
32. Vidor E. Poliovirus vaccine-inactivated. In: Plotkin SA, Orenstein WA, Offit PA, eds. Vaccines 6th Edn). Philadelphia: Elsevier/Saunders; 2013; pp573-597.
33. Hird TR, Grassly NC. Systematic review of mucosal immunity induced by oral and inactivated poliovirus vaccines against virus shedding following oral poliovirus challenge. PLoSPathog 2012; 8:e1002599.
34. Global Polio Eradication Initiative. Polio Eradication & Endgame Strategic Plan 2013-2018. Available from: http://www.polioeradication.org/Portals/0/Document/Resources/StrategyWork/PEESP_EN_US.pdf. Accessed April 24, 2016. .
35. John TJ. Understanding the scientific basis of preventing polio by immunization. Pioneering contributions from India. Proc Indian Natn Sci Acad 2003; B69:393-422.
36. Estivariz CF, Pallansch MA, Anand A, Wassilak SG, Sutter RW, Wenger JD, et al. Poliovirus vaccination options for achieving eradication and securing the endgame. Curr Opin Virol 2013; 3:309-315.
37. Patel M, Zipursky S, Orenstein W, Garon J, Zaffran M. Polio endgame: the global introduction of inactivated polio vaccine. Expert Rev Vaccines 2015; 14:749-762.
38. Anand A, Zaman K, Estívariz CF, Yunus M, Gary HE, Weldon WC, et al. Early priming with inactivated poliovirus vaccine (IPV) and intradermal fractional dose IPV administered by a microneedle device: A randomized controlled trial. Vaccine 2015; 33(48):6816-6822.
39. Resik S, Tejeda A, Sutter RW, Diaz M, Sarmiento L, Alemani N, et al. Priming after a fractional dose of inactivated poliovirus vaccine. N Engl J Med 2013; 368:416-424.
40. Global Polio Eradication Initiative. News Stories ! The Polio Endgame Strategy 2019-2023. Available from: https://polioeradication.org/news-post/the-polio-endgame-strategy-2019-2023/Accessed on 30 September 2020.
41. Kasi SG, Shivananda S, Marathe S, Chatterjee K, Agarwalla S, Dhir S, et al. Indian Academy of Pediatrics (IAP) Advisory Committee on Vaccines and Immunization Practices (ACVIP) Recommended Immunization Schedule (2020) and Update on Immunization for Children Aged 0 Through 18 Years. Indian Pediatrics November 29, 2020 (E-Pub Ahead Of Print).
42. Vidor E. Polio vaccines - Inactivated. In : Plotkin SA, Offit PA, Orenstein WA, Edwards KM (eds). Plotkin's Vaccines. 7th edn. Philadelphia: Elsevier; 2018; pp841-865.
43. Rennels MB. Need for polio boosters after age two years. Vaccine 2009; 27:179-180.
44. National health portal Universal immunization programme; Available from https://www.nhp.gov.in/universal immunisation-programme. Accessed on 30 September, 2020.
45. World Health Organisation. IPV Introduction, OPV Withdrawal and Routine Immunization Strengthening. Available from: http://www.who.int/immunization/diseases/poliomyelitis/endgame_objective2/en/. Accessed on September 14, 2015.

VACCINOLOGY I

ROTAVIRUS VACCINATION

Viswanathan MS

Abstract: *Acute gastroenteritis is one of the leading causes of death in under 5 age group globally. Rotavirus is the most common pathogen causing acute gastroenteritis in children. Rotavirus vaccination has reduced the mortality rate both in low and high income countries. Various high efficacy vaccines are now available. Two indigenously manufactured Indian vaccines are now used in National Immunization Schedule of India. All the currently available vaccines are given orally and safe to use.*

Keywords: *Gastroenteritis, Rotavirus, Rotavirus vaccine.*

Acute gastroenteritis is a leading cause of death in children under 5 years of age and Rotavirus disease resulted in gastroenteritis in millions of children worldwide.[1] Diarrheal diseases account for 5, 25,000 under 5 deaths as per recent data.[1] Prior to the introduction of vaccine, rotavirus disease resulted in 25 million outpatient visits, 2 million hospitalization and 4, 40,000 deaths worldwide in children <5 years of age.[2]

According to Global Enteric Multicenter Study (GEMS), Rotavirus is amongst the four common pathogens that causes moderate to severe diarrhea in South Asia and Sub-Saharan Africa.[3]

Disease burden in India

Diarrheal diseases account for 10% of under-5 mortality in India, resulting in 110,000 deaths in a year.[1,4] In India, rotavirus is responsible for an estimated 11.37 million episodes of acute gastroenteritis in under 5 children annually, resulting in 3.27 million outpatient visits and 872,000 admissions. These account for total direct costs of Indian Rupee (INR) 10.37 billion per year.[5]

* Senior Consultant Pediatric Gastroenterologist
Interventional Endoscopist and
Pediatric Liver Transplant Physician,
Apollo Children's Hospital,
Chennai.
email: vichums@rediffmail.com

Rotavirus Pathogen

The virus was discovered in 1973 with electron microscopy of intestinal biopsies of children with acute gastroenteritis. It is a double stranded RNA virus and belongs to genus Reoviridae. It is classified into seven groups (A to G). Globally, group A is the most important cause for severe acute gastroenteritis in children.[6] VP7 and VP4 proteins form the viral outer capsid. VP7 protein determines the G serotypes and VP4 protein determines the P serotypes (Fig.1). Five G-P combinations account for 90% of circulating human rotaviruses word wide, these include G1 P[8], G2P[4], G3P[8], G4P[8] and G9P[8].[7]

The spread of rotavirus is mainly by person to person contact and it is difficult to control the transmission through improvement in hygiene and sanitation. This is well explained by the disease occurrence universally and with high transmissibility even in those countries with high standards of hygiene. Following rotavirus infection both humoral and cell mediated immunity play a role in protecting the individual. It is usually the homotypic response following the first infection (i.e. against the specific viral serotype). A Mexican study has showed a progressively lower risk of subsequent viral infection following previous infections when compared to those who had no past rotavirus infections.[8] In contrast, an Indian study reported that risk of severe infection exists even after several episodes of past infections and did not find evidence of homotypic protection.[9]

Rotavirus vaccines

The Global Rotavirus Surveillance Network data in 69 predominantly low- and middle-income countries (198 sites) revealed, introduction of rotavirus vaccine (RVV) to be associated with a relative reduction of 40 % (95% CI 35-44 percent) in rotavirus-associated hospitalizations among children <5 year of age.[10]

Attenuation of rotaviruses for use as oral vaccines is usually based on the "Jennerian" concept, involving immunization of infants with animal rotaviruses that are naturally attenuated for humans. Vaccine cost was one of the important factors precluding its use in mid to low income countries like India. Monovalent human bovine

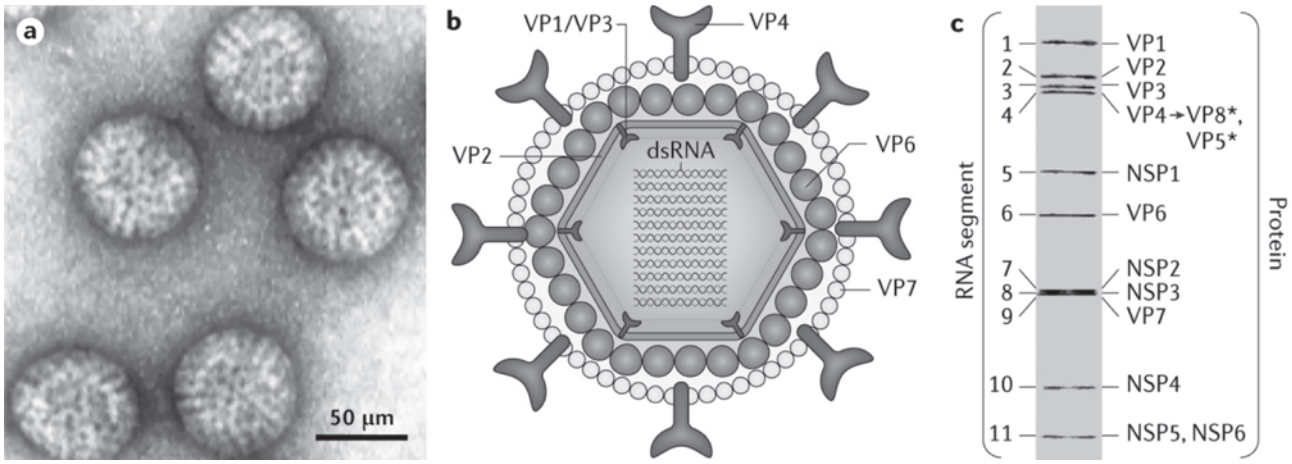

Fig.1. The rotavirus particle resembles a wheel with short spokes and a smooth outer rim. a | Electron micrograph of rotavirus triple-layered particles. b | Cross-sectional schematic of the rotavirus triple-layered particle. This structure consists of the inner capsid layer (viral protein (VP)2), the middle capsid layer (VP6) and the outer capsid layer (VP7 and the spike protein VP4). VP4 is proteolytically cleaved into VP8* and VP5*. The structural protein VP2, the enzymes VP1 and VP3 and the viral genome compose the virion core. The middle capsid layer protein (VP6) determines species, group and subgroup specificities. The outer capsid layer is composed of two proteins, VP7 and VP4, which elicit an immune response in infected hosts, leading to the production of rotavirus-specific antibodies. c | Electrophoretic migration profile of the 11 segments of rotavirus double-stranded RNA (dsRNA) and the encoded proteins for simian rotavirus SA11 strain. NSP, non-structural protein.

Courtesy: Crawford SE, Ramani S, Tate JE, Parashar UD, Svensson L, Hagbom M et al. Rotavirus infection. Nat Rev Dis Primers 2017; 3 (17083):1-16.

Rotavirus vaccine 116E manufactured by Bharat biotech, India and subsequently Bovine rotavirus pentavalent vaccine (BRV-PV) manufactured by Serum Institute of India, were studied in clinical trials and are marketed at a lower cost. The result of Monovalent bovine-human reassortant rotavirus vaccine trial published in 2014 by Bhandari et.al showed the vaccine efficacy of 55.1% against severe gastroenteritis in children up-to 2 years of age (95% CI 39.9 to 66.4) for three doses of vaccine given from 6 weeks of age at 4 weekly interval.[11]

Currently licensed vaccines for use in India

1. Pentavalent human-bovine rotavirus reassortant vaccine (RV5) - Since 2004

It is a human-bovine reassortant vaccine. It contains five reassortant strains between bovine WC23 strain and human G1, G2, G3, G4 and P1A (8). The vaccine induces homologous protection as well as heterologous protection against non-vaccine type G9. Each 2 ml vial contains $2x10^6$ infectious units of each of the five reassortant strains. It is available as liquid vaccine and administered orally.

2. Monovalent human rotavirus vaccine (RV1) - Since 2006

This is a human monovalent vaccine which contains the most common human rotavirus serotype G1P1A (8). It is available as lyophilized powder and one ml contains at-least 10^6 median culture infective units of virus and is administered orally.

3. Human-bovine reassortant vaccine (116E) - Since 2014

It is a monovalent bovine-human reassortant strain vaccine (G9P[11]), one gene is from a bovine-rotavirus strain (P[11]) and 10 genes are from human rotavirus strains. In 1985, the vaccine strain was isolated from asymptomatic infants who presented with mild diarrhea by Indian researchers in New Delhi. Following this isolation, the vaccine development process involved National Institute of Health, USA and Bharat Biotech International Limited, India. It is licensed for use in India and also has World health organization (WHO) pre-qualification for use in developing countries. It is available as liquid vaccine. Single dose is 0.5 ml containing not less than 10^5 FFU (focus forming unit) of live rotavirus 116E and is administered orally.

4. Bovine rotavirus pentavalent vaccine (BRV-PV) - Since 2017

A pentavalent vaccine from five bovine-human reassortant strains that contains serotypes G1, G2, G3, G4 and G9. It was received from US National Institute of Health (NIH) and further developed by Serum Institute of India. It is heat stable and is supplied in freeze dried form. Each dose of 2.5 ml contains NLT $10^{5.6}$ FFU per serotype and is administered orally.

Other vaccines available globally

Human neonatal rotavirus vaccine (RV3-BB)

It was developed from a strain that was isolated from symptomatic neonates, human neonatal rotavirus strain RV3 (G3P[6]), in a nursery. This was administered to neonates and children in a phase 2 trial in Indonesia and this was found to be effective in preventing severe rotavirus gastroenteritis (RVGE) before 18 months of age.[12]

Efficacy and effectiveness of rotavirus vaccines

Jonesteller and his team in a systematic review of rotavirus effectiveness from 2006 to 2016 found that the median effectiveness RV5 is 90% (63 to 100%) in countries with low child mortality (20 studies) and 45% (43 to 92%) in countries with high child mortality (7 studies).[13] Low child mortality countries included in the studies were Australia, Belgium, Canada, Finland, Israel, Portugal, Spain, Taiwan and USA. High child mortality countries were Bolivia, Botswana, El Salvador, Guatemala, Ghana, Malawi, Nicaragua, Rwanda, South Africa, Tanzania and Zambia.

The Rotavirus Efficacy and Safety Trial (REST), which included 68,038 infants from low and high child mortality countries reported protection against rotavirus gastroenteritis of any severity, reduced hospitalization and emergency department visits.[14]

In the Cochrane review (2019) the efficacy and safety of three rotavirus vaccines (RV1, RV5 and 116E vaccines) were analyzed. Fifty-five trials met the inclusion criteria and enrolled a total of 216,480 participants. Thirty-six trials (119,114 participants) assessed RV1, 15 trials (88,934 participants) RV5 and four trials (8432 participants) assessed 116E vaccine.[15]

RV1 in high mortality countries prevents 63% of severe rotavirus diarrhea cases (RR 0.37, 95% CI 0.23 to 0.60; 6114 participants, 3 trials; high-certainty evidence), and 27% of severe all-cause diarrhea cases (RR 0.73, 95% CI 0.56 to 0.95; 5639 participants, 2 trials; high-certainty evidence).

In high-mortality countries, RV5 prevents 57% of severe rotavirus diarrhea (RR 0.43, 95% CI 0.29 to 0.62; 5916 participants, 2 trials; high-certainty evidence), but there is probably little or no difference between vaccine and placebo for severe all-cause diarrhea (RR 0.80, 95% CI 0.58 to 1.11; 1 trial, 4085 participants; moderate-certainty evidence). In a study in India, a high-mortality country, 116E prevents 57% of severe rotavirus diarrhea cases (RR 0.43, 95% CI 0.30 to 0.60; 6799 participants, moderate-certainty evidence); the trial did not report on severe all-cause diarrhea at one-year follow-up.[11] It can be safely said that RV5, RV1 and 116E vaccines prevent significant episodes of diarrhea in high mortality countries though the effect is smaller than the low mortality countries. However, as the baseline risk is higher in high mortality low income countries greater number of episodes are prevented in such settings.

BRV-PV underwent two phase 3 studies in India and Niger. In the Indian study by Kulkarni et al (2017) vaccine efficacy against the very severe rotavirus cases (Vesikari score=16) was 60.5% (95% CI 17.7, 81, p=0.0131) at the time of the primary analysis and 54.7% (95% CI 29.7, 70.8, p=0.0004) for the complete follow up period in the per protocol population. The incidence of severe adverse events, intussusception and deaths were similar between study and control groups.[16]

Risk of intussusception with rotavirus vaccines

Intussusception risk is rare and estimated to occur in approximately 1-2/100,000 vaccinated infants after administration of first dose.[17]

There were 30 cases of intussusception reported in 53,032 children after RV1 vaccination and 28 cases in 44,214 children after placebo or no intervention (RR 0.70, 95% CI 0.46 to 1.05; low-certainty evidence). There were 16 cases of intussusception in 43,629 children after RV5 vaccination and 20 cases in 41,866 children after placebo (RR 0.77, 95% CI 0.41 to 1.45; low-certainty evidence). There were eight cases of intussusception in 5764 children after 116E vaccination and three cases in 2818 children after placebo (RR 1.33, 95% CI 0.35 to 5.02; very low-certainty evidence).[15] Indian studies on 116E and BRV-PV found the risk to be similar in vaccinated and control groups.[11,16] The current generations of rotavirus vaccines are considered quite safe compared to previous vaccine.

In the surveillance for intussusception by African Intussusception Surveillance Network at 28 sentinel

Box 1. Rotavirus vaccination schedule and the dose

- Minimum age: 6 weeks for all vaccines including RV1.
- Maximum age limit for first dose in the series: 14 weeks, 6 days.
- Vaccination should not be initiated for infants aged 15 weeks, 0 days or older.
- Maximum age for the final dose in the series: 8 months, 6 days.
- Interval between doses: 4 weeks
- How many doses?

  RV1: Two doses, with first dose at 6 weeks and second dose 4 weeks later

  RV5, 116E, BRV-PV: Three doses, with first dose at 6 weeks, next 2 doses at 4 weeks interval each.
- Route of vaccination: All RVV to be given orally
- Dose:

  RV1: 1 mL

  RV5: 2 mL

  116E: 0.5 mL

  BRV-PV: 2.5 mL
- Can be given with oral polio vaccine (OPV) and other routine infant immunizations.
- Premature infants: Can be administered RVV if at-least 6 weeks old and clinically stable.
- Interchangeability of RVV: Whenever possible, the vaccine series should be completed with the same product; however, vaccination should not be delayed/ deferred if the product used for previous doses is not available or not known. If any dose in the series was RV5 or an unknown product, a total of 3 doses should be administered.

pediatric hospitals, no increased risk of intussusception was identified after first or second dose of RV1. The Global Advisory Committee on Vaccine Safety (GACVS) of the World Health Organization (WHO) in its report on Jan 2020 concluded that the data on RV5 and 116E did not indicate a significantly higher risk of intussusception during the post-vaccination risk periods than in the reference period for either of the vaccines. Continued monitoring of the risk for intussusception associated with rotavirus vaccines (RVVs) and comparisons of products in the same risk window are recommended when new rotavirus vaccines (RVVs) are introduced into new populations.[18]

In the prospective surveillance for intussusception in India by The International Clinical Epidemiology Network (INCLEN) Intussusception Surveillance Network Study Group, no increase in case load (RR = 0.44; 95% CI 0.22-1.18) or case ratio (RR = 0.5; 95% CI 0.3-1.2) was observed after rotavirus vaccines (RVV) introduction in 19 sentinel hospitals representing all regions of the country.[19]

Recommended schedule for rotavirus vaccines (RVV)

Public health programme

Schedule in Universal immunization program (UIP), India.

In March 2016, the Government of India, introduced the rotavirus vaccines (RVV) (116E) in the universal immunization program (UIP) in a phased manner. The dose of rotavirus vaccine currently being supplied under universal immunization program (UIP) is 0.5 ml given orally. It is administered to all infants at 6, 10 and 14 weeks along with other vaccines in routine immunization schedule.

The upper age limit for giving rotavirus vaccines (RVV) is one year of age as per universal immunization program (UIP). If the child receives the first dose of

Box 2. Contraindications and precautions

Contraindications

- If allergic to any of the ingredients of the vaccine.
- Severe allergic reactions after a previous dose.
- RV1is contraindicated when there is a history of severe allergic reactions to latex. RV1 applicator contains latex.
- History of intussusception.
- Severe combined immunodeficiency (SCID)
- Should be avoided if mother received biologic response modifier in pregnancy. RVV should be delayed for 12 months following the final dose in utero.

Precautions

- Altered immune-competence.
- Moderate to severe illness including gastroenteritis.
- Preexisting chronic gastrointestinal disease.

rotavirus vaccines (RVV) only by 12 months of age, even then 2 further doses need to be given at 4 weeks interval to complete the schedule.[20]

The mortality is highest in children when exposed to rotavirus early in life with lowest levels vaccine coverage and living in poor rural household.[21]

In developing countries as natural infection occurs early in infancy period it is important to complete rotavirus vaccines (RVV) schedule as early as possible to maximize its impact.[17]

Individual use: Rotavirus vaccine schedule and the dose are given in Box 1.[22]

Contra-indications and precautions for the vaccine administration are listed in Box 2.

Points to Remember

- ***Rotavirus vaccines have high efficacy in preventing severe RVGE and rotavirus gastroenteritis associated hospitalizations.***
- ***The current generation of rotavirus vaccines are quite safe.***
- ***It is important to give the first dose at 6 weeks to ensure optimum protection against severe rotavirus gastroenteritis (RVGE) in the vulnerable early infancy period.***
- ***Indigenously manufactured two low cost effective vaccines are available under Universal Immunisation Programme in India.***
- ***Intussusception following rotavirus vaccines is rare. Prospective surveillance has not revealed any increased risk for intussusception in the post-vaccine period, but the surveillance continues.***
- ***It is important to monitor for antigenic/genetic modifications in novel circulating rotavirus strains for which the available rotavirus vaccines may not be effective and for this continued surveillance is necessary.***

References

1. GBD Diarrheal Diseases Collaborators. Estimates of global, regional, and national morbidity, mortality, and etiologies of diarrheal diseases: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Infect Dis 2017; 17(9):909-948.
2. Parashar UD, Hummelman EG, Breeze JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 2003; 9:565-572.
3. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries. The Global Enteric Multicenter Study (GEMS): a prospective, case-control study. Lancet 2013; 382: 209-22.
4. Malik A, Haldar P, Ray A, Shet A, Kapuria B, Bhadana S, et al. Introducing rotavirus vaccine in the Universal Immunization Programme in India: From evidence to policy to implementation. Vaccine 2019; 37:5817-5824.
5. John J, Sarkar R, Muliyil J, Bhandari N, Bhan MK, Kang G. Rotavirus gastroenteritis in India, 2011-2013: Revised estimates of disease burden and potential impact of vaccines. Vaccine 2014; 32S:A5-9.
6. Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. Rev Med Virol 2005; 15:29-56.
7. Matthijnssens J, Van Ranst M. Genotype constellation and evolution of group A rotaviruses infecting humans. Curr Opin Virol 2012; 2(4):426-33.
8. Velazquez FR, Matson DO, Calva JJ, Guerrero L, Morrow AL, Carter-Campbell S, et al. Rotavirus infection in infants as protection against subsequent infections. N Engl J Med 1996; 335:1022-1028.
9. Gladstone BP, Ramani S, Mukhopadhya I, Muliyil J, Sarkar R, Rehman AM, et al. Protective Effect of Natural Rotavirus Infection in an Indian Birth Cohort. N Engl J Med 2011; 365:337-346.
10. Aliabadi N, Antoni S, Mwenda JM, Weldegebriel G, Biey JNM, Cheikh D, et al. Global impact of rotavirus vaccine introduction on rotavirus hospitalisations among children under 5 years of age, 2008-16: findings from the Global Rotavirus Surveillance Network. Lancet Glob Health 2019; 7:E893-E903.
11. Bhandari N, Rongsen-Chandola T, Bavdekar A, John J, Antony K, Taneja S, et al. Efficacy of a monovalent human-bovine (116E) rotavirus vaccine in Indian infants: A randomised, double-blind, placebo-controlled trial. Lancet 2014; 383:2136-2143.
12. Bines JE, At Thobari J, Satria CD, Handley A, Watts E, Cowley D, et al. Human Neonatal Rotavirus Vaccine (RV3BB) to Target Rotavirus from Birth. N Engl J Med 2018; 378(8):719-730.
13. Jonesteller CL, Burnett E, Yen C, Tate JE, Parashar UD. Effectiveness of Rotavirus Vaccination: A Systematic Review of the First Decade of Global Postlicensure Data, 2006-2016. Clin Infect Dis 2017; 65(5):840-850.
14. Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, et al. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. Rotavirus Efficacy and Safety Trial (REST) Study Team. N Engl J Med 2006; 354(1):23-33.

15. Soares-Weiser K, Bergman H, Henschke N, Pitan F, Cunliffe N. Vaccines for preventing rotavirus diarrhoea: vaccines in use. Cochrane Database Syst Rev 2019; 3:CD008521. Epub 2019 Mar 25.
16. Kulkarni PS, Desai S, Tewari T, Kawade A, Goyal N, Garg BS, et al. A randomized Phase III clinical trial to assess the efficacy of a bovine-human reassortant pentavalent rotavirus vaccine in Indian infants. Vaccine 2017; 35(45):6228-6237.
17. World Health Organization. Rotavirus vaccine. Position Paper 2013. Wkly Epidemiol Rec 2013; 88(5): 49-64.
18. Global vaccine safety. Global Advisory Committee on Vaccine Safety, 6-7 December 2017. Available from http://www.who.int/vaccine_safety/committee/reports/Dec 2017.
19. Das MK, Arora NK, Gupta B, Sharan A, Aggarwal MK, Haldar P, et al. Prospective surveillance for intussusception in Indian children aged under 2 years at nineteen tertiary care hospitals. The INCLEN Intussusception Surveillance Network Study Group. BMC Pediatr 2020; 20:413.
20. Operational guidelines. Introduction of Rotavirus vaccine in Universal immunization program in India. Immunization division, Ministry of Health and Family welfare, Government of India. December 2016.
21. Phua KB, Lim FS, Lau YL, Nelson EAS, Huang LM, Quak SH, Lee BW, et al. Rotavirus vaccine RIX4414 efficacy sustained during the third year of life: a randomized clinical trial in an Asian population. Vaccine. 2012; 30:4552-4557.
22. Kasi SG. Rotavirus Vaccines. In: Advisory Committee on Vaccines and Immunization Practices, Indian Academy of Pediatrics. IAP Guidebook on Immunization 2018-2019. Balasubramanian S, Shashtri DD, Shah AK, Pallab Chatterjee, Harish Pemde, Shivananda S, Vijaya Kumar Guduru (eds), 3rd Edn, Jaypee Brothers Medical Publishers, New Delhi, 2020; pp207-226.

CLIPPINGS

Biomarkers in Kawasaki disease

Biomarkers: Several biomarkers have been studied in association with Kawasaki disease (KD) and some of these have been shown to be predictive of resistance to IVIG while others may be indicative of an increased risk of development of coronary artery aneurysms.

They are conventional biomarkers that show inflammatory activity and are largely non-specific as these are also elevated in many other inflammatory conditions. Erythrocyte sedimentation rate (ESR) is consistently elevated during acute phase of KD but it is unreliable after IVIG administration, as a marker of disease activity. C reactive protein (CRP) is said to be associated with disease severity and with development of coronary artery aneurysms. Peripheral blood eosinophilia (PBE) and low albumin levels are associated with increased risk of IVIG resistance and coronary complications. Low mean platelet volume (MPV) and platelet distribution width (PDW) have been shown to be markers of platelet activation and inflammation in acute status of KD.

Immunological Markers: CD14+ monocytes are said to be increased in acute stages of KD and in association with coronary artery abnormalities (CAAs). Whereas, CD8 T cell levels are decreased in acute KD. They are shown to sequester in inflamed coronary arteries and are functionally suppressed. Helper T cells (TH_1 and TH_2) have been shown to be upregulated during acute stages. IL-6 is an important mediator in acute phase, the values are upregulated in acute stages, more elevated in IVIG resistant cases. TNF alpha values are elevated in acute KD and has been shown to have a role in development of CAAs.

Proteomic biomarkers: NT-proBNP is widely believed to be a useful marker for confirmation of diagnosis of KD in the bedside. Higher values as seen in CAAs and can predict IVIG resistance. Cardiac Troponin T, a marker of myocardial damage is elevated in acute stages of KD. Thrombospondin (TSP-1 and the TSP-2) levels are elevated in acute phase and they are associated with high risk of IVIG resistance. Nitric oxide synthases (iNOS) are correlated with severity and progression of CAAs.

Urine protein markers: Filamin, Talin, CSMD3 are non-invasive markers for KD and their levels are higher in acute KD.

Conclusion: The need for a robust set of biomarkers to validate the diagnosis of KD in the clinical setting has become the need of the hour but the clinical application of these markers is still limited and also needs validation across different populations before they are used in confirming the diagnosis of KD in the clinical practice.

Singh S, Jindal AK, Pilania RK. Diagnosis of Kawasaki disease. Int J Rheum Dis. (2018) 21:36–44. doi: 10.1111/1756-185X.13224.

VACCINOLOGY I

CENTRAL NERVOUS SYSTEM VACCINES

**** Aniruddha Ghosh***
***** Ritabrata Kundu***

Abstract: *Vaccines preventing acute central nervous system infections are absolutely essential, because of the high mortality and morbidity associated with these infections. In many viral and bacterial infections, such as pneumococcus, Hemophilus influenzae, mumps, measles and varicella, central nervous system is involved. In this article three important vaccines such as Meningococcal vaccine, Japanese Encephalitis vaccine and Rabies vaccine are covered. Among these, rabies vaccine is also used both as pre and post exposure vaccine.*

Keywords: *Meningococcal vaccine, Japanese encephalitis vaccine, Antirabies vaccine.*

Vaccines against infectious diseases involving the central nervous system are of immense importance.[1] Japanese encephalitis has low infection disease ratio but 60% of the affected will either succumb or have serious neurological sequel.[2] Meningococcal disease in India is relatively low, hence routine meningococcal vaccine is not a priority. However, case fatality rate in meningococcal disease is high and one fifth of the survivors may have permanent disability. Rabies, a viral zoonosis, is uniformly fatal and only few survivors of the disease are reported in world literature.[3] Children in particular have more chance to acquire rabies, hence universal preexposure prophylaxis for them must be considered. Although not in the purview of this chapter, Hemophilus influenzae b and pneumococcal infections, measles, mumps, varicella and tuberculosis are some other vaccine preventable diseases which can present with CNS complications. Japanese encephalitis, meningococcal and rabies vaccines are the central nervous system vaccines discussed here.

Japanese encephalitis vaccine

Japanese encephalitis virus (JEV) is the most common vaccine-preventable cause of encephalitis in Asia (in India contributing around 5-35% of all AES).[4] Transmission principally occurs in rural agricultural areas, often associated with rice cultivation and flooding irrigation. In temperate areas of Asia, transmission is seasonal and human disease usually peaks in summer and fall. In endemic countries, Japanese encephalitis (JE) is primarily a disease of children. However, travel-associated JE can occur among people of any age. JE has around 30% case fatality and 30% to 40% of survivors suffer from long term neurological sequelae and morbidity.[5] In endemic areas there are few human cases among residents because of vaccination or natural immunity. Japanese encephalitis virus is often still maintained in an enzootic cycle between the natural hosts (pigs and birds, particularly cattle egrets, pond herons, etc.)[1] and mosquitoes.[4] Due to widespread vaccination, currently JE has come down in the endemic states of India.

World over, several vaccines are available against JE. Mouse brain-derived inactivated JE vaccine (JE-VAX) and inactivated primary hamster kidney cells with P3 vaccine made in China are not used nowadays. Licensed vaccines available in India are:

- Inactivated SA 14-14-2 vaccine (JEEV® by Biological E Ltd.)
- Inactivated Vero cell-culture derived Kolar strain, 821564XY, JE vaccine (JENVAC® by Bharat Biotech.)
- Live attenuated SA 14-14-2 vaccine made by Chengdu Institute of Biological Products Co. Ltd. (People's Republic of China)

Routine vaccination

- Recommended for individuals up to 18 years of age living in endemic areas.
- Live attenuated, cell culture derived SA14-14-2 (SA 14-14-2 JE vaccine is a live, attenuated vaccine given subcutaneously).
  - Minimum age: 8 months
  - Two dose schedule
    - First dose at 9 months along with measles, rubella (MR) vaccine

* Assistant Professor,
** Professor, Department of Pediatrics, Institute of Child Health, Kolkata.
email: rkundu22@gmail.com

- Second at 16 to 18 months along with DTP booster, MR second dose
- Stability: Very stable vaccine; retains infectious titre even at room temperature for 4 months and at 2-8 degree C for 1.5 years.
- Immunogenicity: After a single dose, antibody response is produced in 85-100% of non immune children.
- Efficacy and effectiveness: Multiple trials and case control studies conducted in China showed an efficacy of at least 95%, following two doses administered 1 year apart. Indian scenario is slightly different. A case control study conducted by ICMR showed an unadjusted protective effect of 62.5%. According to this result, the efficacy was around 60% in Uttar Pradesh and around 70% in Assam.[4,5]
- Safety: WHO has acknowledged the vaccine as "excellent" with respect to its safety.[6] Minor local reactions including transient fever have been noted in very small percentage of children. Neither encephalitis nor serious hypersensitivity reactions have been reported with the use of this vaccine.
- India has been using this vaccine since 2006 in selected JE endemic districts as integral part of the National Immunization Programme.
- Not available in private market for office use.

- **Inactivated cell culture derived SA14-14-2 (JEEV® by BE India)**
  - Minimum age: 1 year
  - Primary immunization schedule: 2 doses of 0.25ml each administered intramuscularly on days 0 and 28 for children aged 1-3 years
  - 2 doses of 0.5 ml for children >3years, adolescents and adults
  - Need of boosters still undetermined
- **Inactivated vero cell culture-derived Kolar strain, 821564XY, JE vaccine (JENVAC® by Bharat Biotech)**
  - Minimum age: 1 year
  - Primary immunization schedule: 2 doses of 0.5 ml each administered intramuscularly at 4 weeks interval
  - Need of boosters still undetermined.

Catch up vaccination

- All susceptible children up to 18 years should be administered during disease outbreak/ahead of anticipated outbreak in campaigns.

Public health aspect

Vaccination of susceptible population is the only means to prevent JE. All the Global JE meetings held in 1995, 1998 and 2002 agreed upon the fact that it is the only long term measure that can be taken against JE. Though in India rural children are mostly affected, urban cases have also been reported. That's why Govt. of India has taken the decision to routinely vaccinate all rural and urban children as a commitment against JE.

Japanese encephalitis has epidemic potential in India and has a high case fatality. Surveillance is important to detect actual disease burden and early warning signals for predicting JE outbreak and to initiate timely effective control measures. Till 2005, JE was reported as suspected JE; however, as per the revised guidelines prepared by National Vector Borne Disease Control Programme, JE is being reported under the umbrella of AES. Japanese encephalitis surveillance system collects the information on epidemiologic,clinical, laboratory and entomological parameters from the identified sites on a regular basis.[5,7]

Meningococcal vaccine (MCV)

Neisseria meningitides (Meningococcus) meningitis is a rare endemic disease in most countries; hyperendemic and epidemic patterns are also noted sometimes. There are 13 known serotypes of meningococci and 6 of them (A, B, C, Y, X and W-135) are associated with most of the epidemics. Since early 2000, incidence of invasive meningococcal disease (IMD) is steadily declining. Serotype B has more predilection for children below 5 years of age, while serotypes C, Y and W affects children mostly above 11 years age.[8-9] India has had serogroup A epidemics in Delhi and Northeast India but burden of IMD in non-outbreak settings in India is very low. A 10-years retrospective analysis of acute bacterial meningitis from Bangalore demonstrated that meningitis due to meningococci only accounted for 1.4% of culture proven cases.[10] According to Ministry of Health and Family Welfare (MoHFW), IMD is a notifiable disease in India and currently the incidence is declining in our country.[9,11] Hence meningococcal vaccine has not been placed in the list of routine immunization.

Two types of meningococcal vaccines are available worldwide.[12]

1. Meningococcal polysaccharide vaccine (MPSV4)

- Either bivalent (A, C) or quadrivalent (A, C, Y, W-135).
- To be stored at 2-8 deg C.
- T cell independent response, hence do not induce immunological memory.
- Antibody response to each of the four polysaccharides is independent and serogroup specific.
- Serogroup A and C vaccines have good immunogenicity and show clinical efficacy of more than 85% in children older than 5 years.
- Serogroup Y and W containing vaccines are also safe and immunogenic in older children and adults.
- In children less than 5 years of age, antibody titres fall substantially in first 3 years post vaccination.
- In adults after administering subsequent boosters, hyporesponsiveness has been documented.
- Vaccine is safe and has shown only local side effects like pain, redness, etc.

2. Meningococcal polysaccharide-protein conjugate vaccine

(i) Quadrivalent Meningococcal Polysaccharide-protein Conjugate Vaccine/MCV4

(MenACWY-D, Menactra®, Manufactured by Sanofi Pasteur)

- Licensed in India in 2012 for age group 2-55 years.
- It contains 4 µg each of A, C, Y, W-135 polysaccharide conjugated to 48 µg of diphtheria toxin which is the carrier.
- A single 0.5 ml intramuscular dose is recommended.
- Within 3-4 years of immunization, effectiveness was found to be 80-85%.
- Interferes with PCV13, so interval of 4 weeks should be kept between these two vaccines and PCV13 is to be given first.
- Some cases of Guillain Barre Syndrome have been observed in adolescent vaccine recipients but causal association has not been proved.
- Otherwise the vaccine is safe and causes only minor local reactions.

(ii) Monovalent Serogroup A Conjugate vaccine

(PsA–TT, MenAfriVac®, Manufactured by Serum Institute of India)

- It is a monovalent serogroup A polysaccharide vaccine conjugated to tetanus toxoid.
- Alum as adjuvant and thiomersal as preservative are added to it.
- The vaccine was licensed in India in 2009 and prequalified by WHO in 2010, but still not readily available in India for office use.
- Single 0.5 ml intramuscular dose is recommended in the age group of 1-29 years.
- This vaccine is safe and has got great effectiveness giving a long lasting immunity as evidenced by studies conducted in Africa.

(iii) A quadrivalent conjugate meningococcal vaccine (Glaxo SmithKline) has been recently licensed by the Drug Controller General of India. This vaccine has been proven safe, efficacious and non-inferior to Menactra.

Both MPSV4 and MCV4 give protection against serotypes A, C, Y and W. MPSV4 has capsular polysaccharide antigens and licensed for use in children older than 2 years of age. Conjugate vaccine due to its greater immunogenicity and lesser hyporesponsiveness is preferred over polysaccharide vaccine.

Indian Academy of Pediatrics (IAP) has recommended meningococcal vaccines for certain patients/conditions.[13]

During meningococcal outbreaks: Polysaccharide vaccines can be used when the supply of MCVs is scarce or due to economic reasons. However, due to lesser immunogenicity and limited efficacy of polysaccharide vaccines in children younger than 2 years of age, MCV is the preferred choice for protection in this age group and particularly for MenA disease. Monovalent MCV against MenA like PsA-TT should be employed for mass vaccination, as in India most sporadic outbreaks are due to MenA.

Persons with high-risk conditions/situations

(i) Terminal complement component deficiencies

- 2-dose primary series of MCV recommended in persistent deficiencies.
- Recommended age group: 24 months - 55 years.
- A booster dose is recommended once in every 5 years.
- Children receiving primary series before their 7th birthday should be given first booster after 3 years and subsequent boosters every 5 years.

(ii) Functional/Anatomic asplenia/hyposplenia

- 2-primary doses of either MCV
- The interval between 2 doses should be minimum 8 weeks
- Recommended age group: 24 months-55 years
- Vaccination should be started 2 weeks prior to splenectomy.

(iii) Persons with human immunodeficiency virus (HIV) infection

- 2 doses at least 8 weeks apart.

(iv)Laboratory personnel and healthcare workers

- Those lab workers who are at risk of exposure to meningococci in aerosols.
- Single dose MCV recommended
- In case of continuous exposure, booster is to be administered once in every 5 years

(v) Adjunct to chemoprophylaxis

- Close contacts of patients with meningococcal disease
- Single dose appropriate group MCV is recommended.

International travellers

(i) Students going for study abroad

Some institutions require vaccination of students against meningococci during admission. Persons aged less than 21 years should have a documented proof of administration of MCV within the last 5 years. American Academy of Pediatrics recommends routine MCV at age 11-12 years and a booster at age 16 years.

(ii) Hajj pilgrims

- Vaccination within 3 years before the date of travel is necessary for all travellers to Mecca during Hajj.
- Quadrivalent vaccine providing protection against emerging W and Y serotypes in these areas.
- Single dose 0.5 ml IM is recommended for 2-55 years age group.

(iii) Travellers to countries in African meningitis belt

- Single dose monovalent/quadrivalent vaccine is recommended.
- Booster dose MCV is required if the last dose was received 5 or more years prior..

Anti rabies vaccine (ARV)

Rabies virus is a single stranded RNA virus and it causes severe encephalitis with fatal outcome. This zoonotic disease is transmitted to humans mostly by bite or scratch of an infected animal (in India in 95% cases by infected dogs) or by contact of saliva with broken skin or mucous membranes in the eyes, nose, or mouth. Once the symptoms start, it is irreversible. The virus multiplies in striated muscles and gradually ascends through axons of peripheral nerves to spinal cord and ultimately to brainstem, medulla and pons destroying neurons extensively causing death. This disease is 100% preventable if timely prophylactic measures are taken. Anti rabies vaccine (ARV), rabies immunoglobulin (RIG) or rabies monoclonal antibody are the mainstay of prophylaxis in addition to wound care.

ARV prophylaxis

- To protect those at risk of exposure to rabies virus (Pre-exposure vaccination).
- To prevent development of clinical disease after exposure to virus (post-exposure prophylaxis).

The vaccines available for both pre and post-exposure vaccination are the same, but the schedule differs. Prophylaxis should start as soon as the exposure occurs. Active immunization is achieved by administration of potent and safe ARVs. The vaccines available now are the modern cell culture vaccines (CCVs).[14]

Types of CCVs

1. Cell culture vaccines

- Human diploid cell vaccine (HDCV)
- Purified chick embryo cell vaccine (PCECV)
- Purified Vero cell rabies vaccine (PVRV)

2. Purified duck embryo vaccine (PDEV)

Irrespective of age and weight of the child the schedule, dosage etc. are same for all the vaccines.

Storage and transportation

It is recommended that the vaccines should be kept and transported at a temperature range of 2-8 degree C. They should never be frozen.

Reconstitution and storage

The lyophilized vaccine has to be diluted with diluents provided in the pack just before use and it should be discarded after 6-8 hours after reconstitution if not used.

Adverse effects

CCV and PDEV are safe and usually do not cause any significant side effects apart from pain and tenderness at the injection site. Rarely systemic side effects i.e. fever, malaise, hypersensitivity reactions etc can occur. In such circumstances chick embryo vaccines should be replaced by Vero cell derived one and vice versa.

Exchange of vaccine brands

Switching over from one type/brand to other is not recommended but if one type/brand is not at all available then the schedule should be completed with any other type/brand of vaccine available. There is no need to restart the schedule.

Co administration

Rabies vaccines can safely be given with other killed and live vaccines, using different injection sites for each vaccine.

Pre-exposure vaccination

Indicated before exposure to virus.

Number of doses: Three, one on each of days 0, 7 and 21 or 28, given intramuscular (1 or 0.5 ml/dose depending on the vaccine) or intradermal (0.1 ml/inoculation site).

Booster: Not routinely needed for general travellers.

Post-exposure prophylaxis (Table I)

- Cell-culture- or embryonated-egg-based rabies vaccines should always be used for post-exposure prophylaxis.
- Bite by wild animals should be treated as category III.
- Vaccination status of the biting animal and whether the bite was provoked or not does not change the course of PEP.
- If the dog or cat is observed for 10 days and it remains healthy, then PEP can be modified accordingly. It doesn't apply to other animal bites.
- Drinking milk of a rabid cow or breastfeeding from a rabid human is not an indication for PEP.

Intramuscular regimens

Both a five-dose and a four-dose IM regimen (where more rapid antibody response is needed) are recommended by WHO for post-exposure vaccination; the five dose regimen is currently approved for use in India:

- The five-dose regimen is administered on days 0, 3, 7, 14 and 28 into the deltoid muscle or lateral aspect of thigh.[15]
- The four-dose regimen is administered as two doses on day 0 (one dose in the right and one in the left arm (deltoid muscles of both arms simultaneously) and then one dose on each of days 7 and 21 into the deltoid muscle or lateral aspect of thigh.

For children less than 2 years age, anterolateral aspect of thigh and for all patients aged more than 2 years, deltoid muscle are the WHO recommended sites for IM injection. Gluteal muscle should never be used as absorption from this region is erratic.

An alternative post-exposure regimen for healthy, fully immunocompetent exposed people who receive wound care plus high-quality rabies immunoglobulin plus WHO-prequalified rabies vaccines consists of four doses administered IM on days 0, 3, 7 and 14.[16]

Intradermal regimens

Intradermal administration of cell-culture- and

Table I. Type of contact, exposure and recommended post exposure prophylaxis[14]

Category of contact	Type of exposure	Recommended post exposure prophylaxis
I. Touching or feeding of animals Lick on intact skin	None	None, if history is reliable
II. Nibbling of uncovered skin Minor scratches, abrasions without bleeding	Minor	Wound management + ARV
III. Single or multiple transdermal bites or scratches, licks on broken skin Contamination of mucous membrane with saliva (i.e. licks)	Severe	Wound management + RIG/Monoclonal antibody +ARV

embryonated-egg-based rabies vaccines has been successfully used in many developing countries as it is equally protective as the five- or four-dose IM schedules and is cost-effective.

The two-site ID method: One ID injection at two sites on days 0, 3, 7 and 28 (2-2-2-0-2). No injection on day 14. The volume per intradermal injection should be 0.1 ml with both purified Vero cell and purified chick embryo rabies vaccine.

Some points regarding intradermal rabies vaccination (IDRV)

- ID injections require trained personnel.
- Vaccines must be stored at 2-8 degree C after reconstitution (all the more important as it is a multidose vaccine).
- Reconstituted vaccine must be used within 8 hours or else discarded.
- For IRDV Drug Controller General of India (DCGI) approved vaccines only are to be used.
- Vaccines given ID should raise a palpable and visible bleb of 3-4 mm size.
- If any dose is inadvertently given in subcutaneous or intramuscular route another dose should be given properly in nearby region, intradermally.
- If IDRV schedule has been discontinued, it should be resumed.
- ID injections should be given in the deltoid, anterolateral thigh or suprascapular regions.[14]

For repeat exposure

Irrespective of duration of previous post-exposure prophylaxis, 2 doses of ARV given in ID/IM route is recommended on Day-0 and Day-3. Proper wound care needs to be practised and RIG is not required.

For immunocompromised children

PEP as for normal children is to be given. Thorough wound cleaning is of utmost importance in case of immunocompromised children. Rabies virus neutralising antibody titre should be checked 2-4 weeks after completion of PEP to determine whether any additional dose of vaccine is necessary.

Points to Remember

- ***Many bacterial or viral infections can lead to CNS infections or complications related to CNS.***
- ***JE disease carries a high risk of mortality of around 30% and 30% to 40% of survivors suffer from long term neurological sequelae and morbidity, hence JE vaccination is essential for children and adolescents living in endemic areas.***
- ***Because of the intense vaccination, strategies currently the incidence JE has been drastically reduced in the endemic states of India.***
- ***Meningococcal vaccine has not been placed in the list of routine immunization. But is being used for specific purposes like travelling abroad.***
- ***Antirabies vaccine has been used both as pre and post exposure vaccine. Site of vaccination is important and it is given in deltoid region as well as in the lateral aspect of thgh. It should never be given in the gluteal region.***
- ***In class III exposure, rabies immunoglobulin or monoclonal antibody has to be given in addition to antirabies vaccine.***
- ***Antirabies vaccine as ID injections required to be given only by trained personnel, in the deltoid, anterolateral thigh or suprascapular regions***

References

1. Leibovitch EC, Jacobson S. Vaccinations for neuroinfectious disease: A global health priority. Neurotherapeutics 2016; 13(3):562-570.
2. Tiwari S, Singh RK, Tiwari R, Dhole TN. Japanese encephalitis: A review of the Indian perspective. Braz J Infect Dis 2012; 16:564-573.
3. Tarantola A. Four thousand years of concepts relating to rabies in animals and humans, its prevention and its cure. Trop Med Infect Dis 2017; 2(2):5.
4. Janowski AB, Hunstad DA. Viral meningoencephalitis. In: Kliegman RM, Geme III JW, Blum NJ, Shah SS, et al. Nelson Textbook of Pediatrics, 21st edn. Philadelphia: Elsevier; 2019; pp3232-3234.
5. Immunization Division Department of Family Welfare Ministry of Health and Family Welfare, Government of India. Control of Japanese Encephalitis. Operational Guide Japanese Encephalitis Vaccination in India. 2010; pp13-15. Available from: (https://nvbdcp.gov.in/Doc/JE-AES-Prevention-Control(NPPCJA).pdf Accessed 12 Oct, 2020)
6. World Health Organization. Japanese Encephalitis Vaccines: WHO position paper February 2015-Recommendations. Vaccine 2016; 34:302-303.
7. Bista MB, Banerjee MK, Shin SH, Tandan JB, Kim MH, Sohn YM, et al. Efficacy of single-dose SA 14-14-2 vaccine against Japanese encephalitis: a case control study. Lancet 2001; 358:791-795.

8. American Academy of Pediatrics. Meningococcal Infections. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS (Eds). Red Book: 2012 Report of the Committee on infectious Diseases. Elk Grove Village, IL: American Academy of Pediatrics; 2012; pp500-509.
9. CDC. VPD surveillance manual, MacNeil J, Patton M. Chapter 8: Meningococcal Disease. [online] Available from: https://www.cdc.gov/vaccines/pubs/surv-manual/chpt08-mening. pdf Last accessed on 16 September, 2020.
10. Mani R, Pradhan S, Nagarathna S, Wasiulla R, Chandramuki A. Bacteriological profile of community acquired acute bacterial meningitis: a ten-year retrospective study in a tertiary neurocare centre in South India. Indian J Med Microbiol 2007; 25(2):108-114.
11. Central Bureau of Health Intelligence. 2016. National Health Profile, India 2012-2016. Available from: www.cbhidghs.nic.in Last accessed on 16th September, 2020.
12. World Health Organization (WHO). Global Advisory Committee on Vaccine Safety, 9-10 June 2005. Wkly Epidemiol Rec 2005; 80:242-243.
13. Balasubramanian S, Shah A, Pemde HK, Chatterjee P, Shivananda S, Guduru V, et al. Indian Academy of Pediatrics (IAP) Advisory Committee on Vaccines and Immunization Practices (ACVIP) Recommended Immunization Schedule (2018-19) and Update on Immunization for Children Aged 0 Through 18 Years. Indian Pediatr 2018; 55(12):1066-1074.
14. World Health Organization. Rabies vaccines: WHO Position Paper, April 2018 Recommendations. Vaccine 2018; 36:5500-5503.
15. Ministry of Health and Family Welfare. Government of India. National Guidelines for Rabies Prophylaxis, 2019. National Rabies Control Programme. Available from: https://www.nhp.gov.in/disease/neurological/rabies accessed 13 October 2020.
16. Rupprecht CE, Briggs D, Brown CM, Franka R, Katz SL, Kerr HD, et al. Use of a reduced (4-dose) vaccine schedule for postexposure prophylaxis to prevent human rabies: recommendations of the advisory committee on immunization practices. MMWR Recomm Rep 2010; 59(RR-2):1-9.

CLIPPINGS

Serum ferritin as a marker to predict the severity of dengue fever

Annually close to 10 lakh dengue cases are reported to the WHO, which in the tropical and subtropical regions have proven to be a paramount cause of morbidity. The illness comprises of 3 phases: febrile, critical followed by recovery. Clinically, dengue can be classified as - Dengue without warning signs, Dengue with warning signs and Severe Dengue. The clinical spectrum of dengue infection is variable from mild fever to severe forms of dengue such as dengue hemorrhagic fever and dengue shock syndrome. Severe dengue is characterized by severe thrombocytopenia with major bleeding, plasma leakage resulting in fluid accumulation, respiratory distress and multi-organ dysfunction.

Clinically severe dengue results from interplay between virus related virulence factors and host factors which include inflammatory response of the host to infection with exuberant T and B cell activation, release of cytokines (cytokine storm), altered endothelial function with increased vascular permeability and nutritional status of the host.

Increased serum ferritin has been associated with severe dengue in children. Ferritin, is an acute phase reactant and hyperferritinemia is a hallmark of diseases which occurs owing to an extensive immune activation. Ferritin is produced by reticuloendothelial cells in response to inflammation and infection. Amid a dengue outbreak in Aruba, on the Caribbean island, raised ferritin level was a finding in those who were suffering from dengue virus infection. An Indian study had shown that serum ferritin level are significantly elevated in dengue infected cases compared to the controls. On group analysis, cases with severe dengue had higher ferritin levels than milder forms which was noted both during the febrile and defervescence stages of the illness. Results from the previous studies had also shown that hyperferritinemia is associated with severe disease which is noted throughout the disease course. Mean ferritin levels are also high in patients with severe dengue as compared to dengue fever. A study conducted in Tamil Nadu by Petchiappan V et al, stated that elevated serum ferritin levels done early during the febrile stage of the illness (3rd to 7th day of illness) can predict the severity of dengue. In another South Indian study performed by Soundravally R, et al, hyperferritinemia (Ferritin >500 ng/mL) emerged a strong predictor of severe dengue and worsening thrombocytopenia.

Petchiappan V, Hussain TM, Thangavelu S. Can serum ferritin levels predict the severity of dengue early? : An observational study. Int J Res Med Sci. 2019 Mar; 7:876-881.

VACCINOLOGY I

COLD CHAIN - MAINTENANCE AND MONITORING

* ***Srinivas G Kasi***

Abstract: *The cold chain, also known as the immunization supply chain, is the lifeline of any immunization program. It is a system of storing and transporting vaccine at the recommended temperature range from the point of manufacture to point of use. The main components are personnel, equipment and protocols. The cold chain equipment in use are the domestic refrigerators, ice-lined refrigerators and the purpose-built refrigerators. Temperature monitoring devices include the vaccine vial monitors, thermometers, data loggers and freeze indicators. Passive storage devices include vaccine carriers and cold boxes. Vaccines should be stored in a recommended manner for optimal storage and maintenance of the recommended temperature range. New technologies and innovations are being harnessed to improve the performance of the cold chain system.*

Keywords: *Vaccine, Cold chain.*

There are few immunization issues more important than the appropriate storage and handling of vaccines. Cold chain is a system of storing and transporting vaccines at the recommended temperature range from the point of manufacture to point of adminstration (Fig.1). The cold chain is also termed as "immunization supply chain".[1] Vaccines are biological products and hence are sensitive to heat, cold and sunlight. Storage under optimum conditions is essential for maintenance of potency throughout the shelf life. Improperly stored vaccines may lose potency and result in vaccine failures which may have a negative impact on the immunization program, apart from incurring significant financial losses. Loss of potency is an irreversible process.

Vaccines must be stored properly from the time they are manufactured until they are administered. A proper cold chain is a temperature-controlled supply chain that includes all equipment and procedures used in the transport and storage and handling of vaccines from the time of manufacture to administration of the vaccine. This could be a long journey, across oceans and countries, with intermediate storage facilities of varying capacities and equipment requirements (Fig.1).

* Consultant Pediatrician,
Convener, ACVIP 2020-21
email: sgkasi@gmail.com

An effective cold chain relies on three main elements[2]

- Well-trained staff to manage vaccine storage and distribution.
- Reliable storage and temperature monitoring equipment.
- Efficient management procedures for optimum utilization of equipment, accurate vaccine inventory management

Thermostability of vaccines

Generally, live-attenuated vaccines are heat sensitive while the inactivated vaccines are less heat sensitive but more freeze intolerant.

Both, freezing and warming, are equally harmful and common. The WHO has categorized vaccines into 6 classes based on their relative heat sensitivities (Fig.2).[3]

It should be noted that this categorization applies only for freeze-dried vaccines which are unopened vials. Reconstitution leads to fairly rapid loss of potency (Box 1).

Although the thermostability of vaccines varies by vaccine type, in general all vaccines are to be stored at a temperature between $+2^0$ C and $+8^0$ C. Some vaccines are sensitive to light and are supplied in amber colored vials. These include Measles/MR/MMR, BCG, rota virus vaccine (RVV) and Japanese encephalitis (JE) vaccines.

Cold chain equipment (CCE) used for storing and transporting vaccines are of two types - active systems and passive systems.[4] Active systems consist of CCE which operate on electricity obtained from a power grid and off-grid refrigerators which run on either LPG / kerosene or solar power generated electricity. Passive systems consist of cold boxes and vaccine carriers, wherein there is no active refrigeration mechanism, but cooling is achieved by coolant packs.

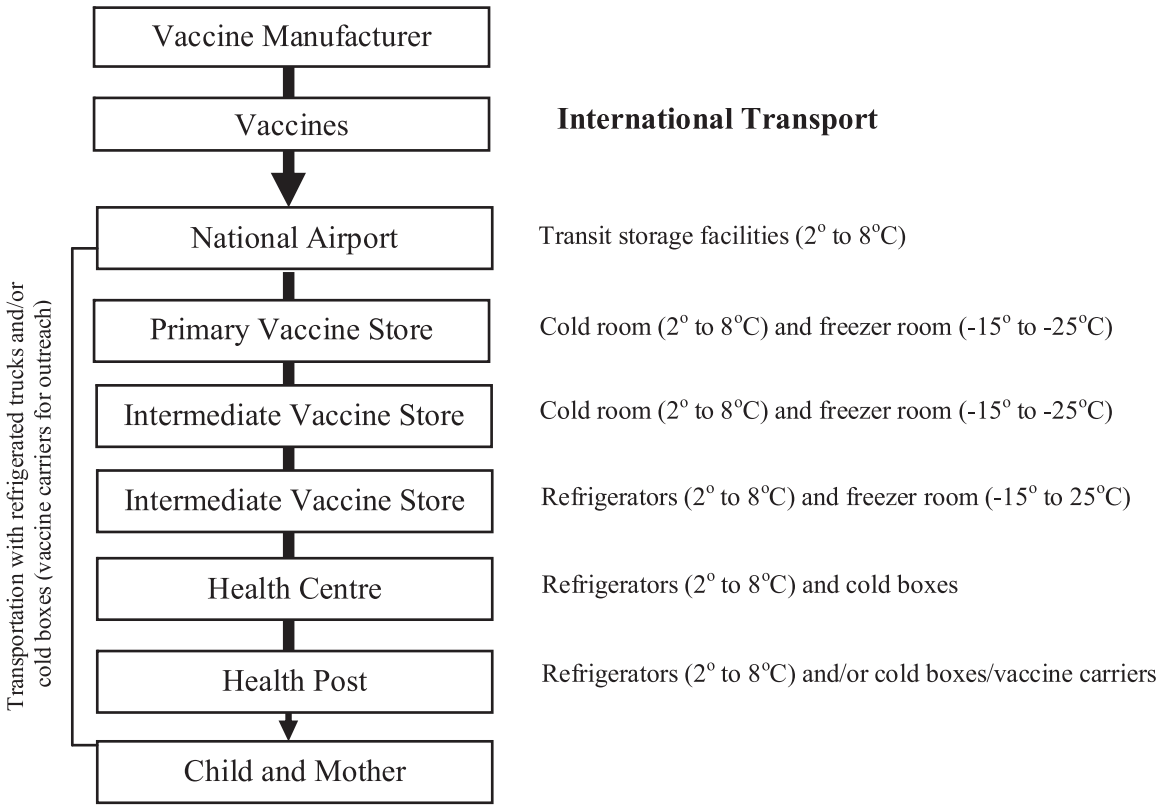

Fig.1. Recommended temperature ranges from the point of manufacture to point of administration

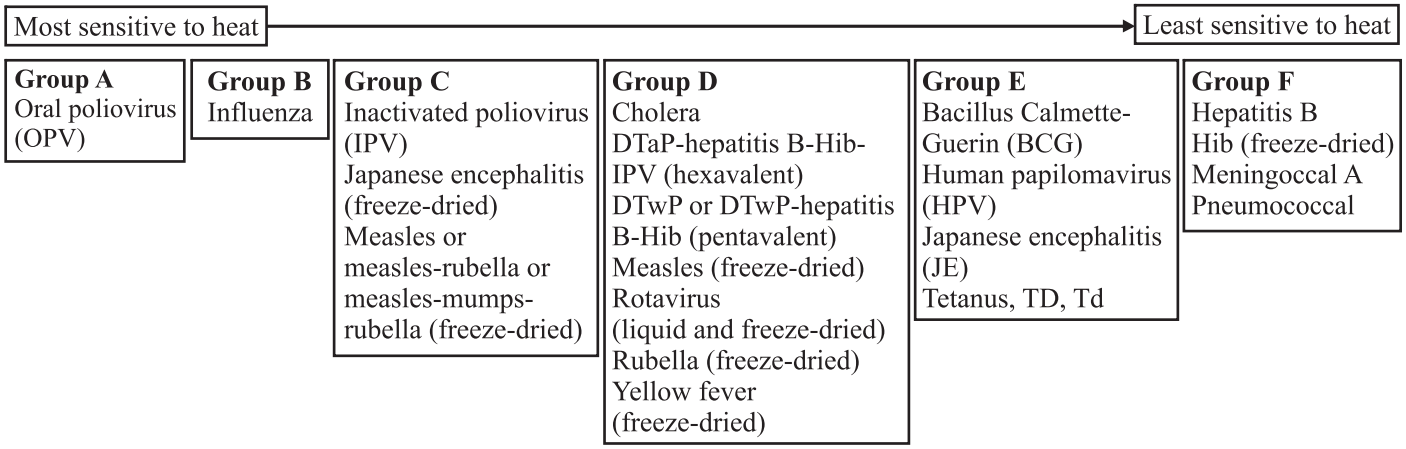

Fig.2. WHO categorization of vaccines according to thermostability

Electrical cold chain storage equipments in the UIP in India include cold rooms (Government medical store depots), walk-in coolers (WIC) and walk-in freezers (WIF) in the regional and state vaccine stores, ice-lined refrigerators (ILRs) and deep freezers (DF) in the district, divisional stores and the primary health centers.[5]

Vaccine management protocol

A vaccine management protocol should be in place.[6]

This includes:

- A trained, designated person and a trained backup person, who will be responsible for vaccine storage and follows the recommended protocols.

Box 1. and Box 2. Categorization of vaccines based on sensitivity to heat and freezing

Vaccines sensitive to heat

Most sensitive

BCG (after reconstitution)
OPV
Measles / MMR
(Before and after reconstitution)
DPT
BCG (before reconstitution)
HIB
Td
TT
Hepatitis B

Least sensitive

Vaccines sensitive to freezing

Most sensitive

Hepatitis B
PCV
Penta
IPV
DPT
TT / TD

Least sensitive

freeze dried live attenuated vaccines are least sensitive to freezing.

- All staff members involved in handling vaccines should be well versed in these protocols.
- Contact names and numbers for reporting
  - cold chain breaches
  - refrigerator and/or logger maintenance issues
  - power failures
- Back up vaccine storage options

Electrical equipment for storage of vaccines in clinics

1. Domestic refrigerators
2. Purpose built refrigerators
3. Ice-lined refrigerators

Domestic refrigerators

Domestic refrigerators are the commonest equipment used for storage of vaccines in clinics and hospitals. It should be noted that these are not technically designed to store vaccines and biologicals. The disadvantages include.[6]

- Excursions beyond the safe range of $+2^0$ C to $+8^0$ C are not uncommon.
- Cyclic defrost refrigerators are not recommended as they have wide temperature fluctuations.
- Most domestic refrigerators have poor temperature recovery after the door has been opened.
- Most domestic refrigerators are affected by the ambient temperature.

The WHO does not recommend domestic refrigerators for vaccine storage even in the clinic setting.

There are 3 types of domestic refrigerators: Cyclic defrost, manual defrost and auto defrost. In the first 2 types, during the defrost process, the temperature excursions may occur beyond the safe zone and hence are not recommended for vaccine storage.

Bar-type refrigerator and single door refrigerator are not recommended due to the risk of freezing of vaccines.[1] Direct cool refrigerators are to be avoided as there is uneven temperature distribution and formation of ice from the water vapor inside the refrigerator.

The domestic refrigerator chosen should have separate doors for the freezer and main compartments, frost free type, large enough to store a month's supply of vaccines. A built in thermometer with a temperature display on the door is preferred.

Placement of cold storage equipment[7]

- The unit should be placed in a well-ventilated room, away from direct sunlight and not adjoining an outside wall.
- At least 10 cm of space should be maintained between the sides of the equipment and surrounding walls, to enable air circulation all around.
- The coils and motor compartment should not be blocked by anything.
- The unit should stand firmly and level. The wheels or leveling legs should be adjusted so that the bottom of the unit sits 2.5 to 3 cm above the floor.

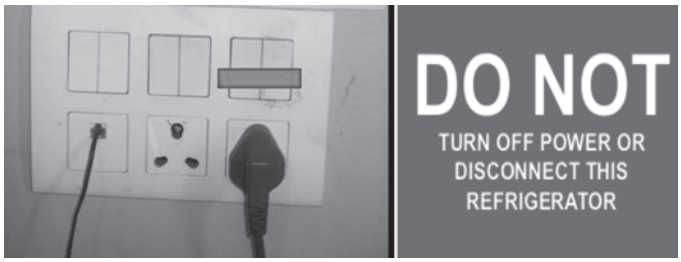

Fig.3. Electric socket

- The door should auto-close.
- The electric socket should be switchless or the switch should be taped to avoid accidental switching off. (Fig.3**)**

Setting and stabilizing the temperature: [6]

- Switch on the power source. Check the temperatures inside the refrigerator and freezer compartments.
- Adjust the temperature indicator slightly toward a warmer or colder setting depending upon the temperature recorded.
- Allow the temperature inside the unit to stabilize for half an hour then recheck the temperature.
- Adjust the thermostat again as necessary till a temperature of +5 ^{0}C is recorded in the main compartment and -15^0 C in the freezer compartment.
- In a new refrigerator, allow one week of twice daily refrigerator and freezer temperature recordings before using the unit to store vaccines.
- Avoid unnecessary opening of the refrigerator door. The WHO recommends door openings be minimized to not more than four times a day.

Short lasting temperature variations do not warrant changes in the thermostat settings, whereas variations lasting for a few hours or gradually moving towards the outer limits, warrant change in the thermostat settings.

Storing vaccines in the refrigerator

Since cooling occurs from the freezer in the top, temperatures are least in the upper shelves and slightly

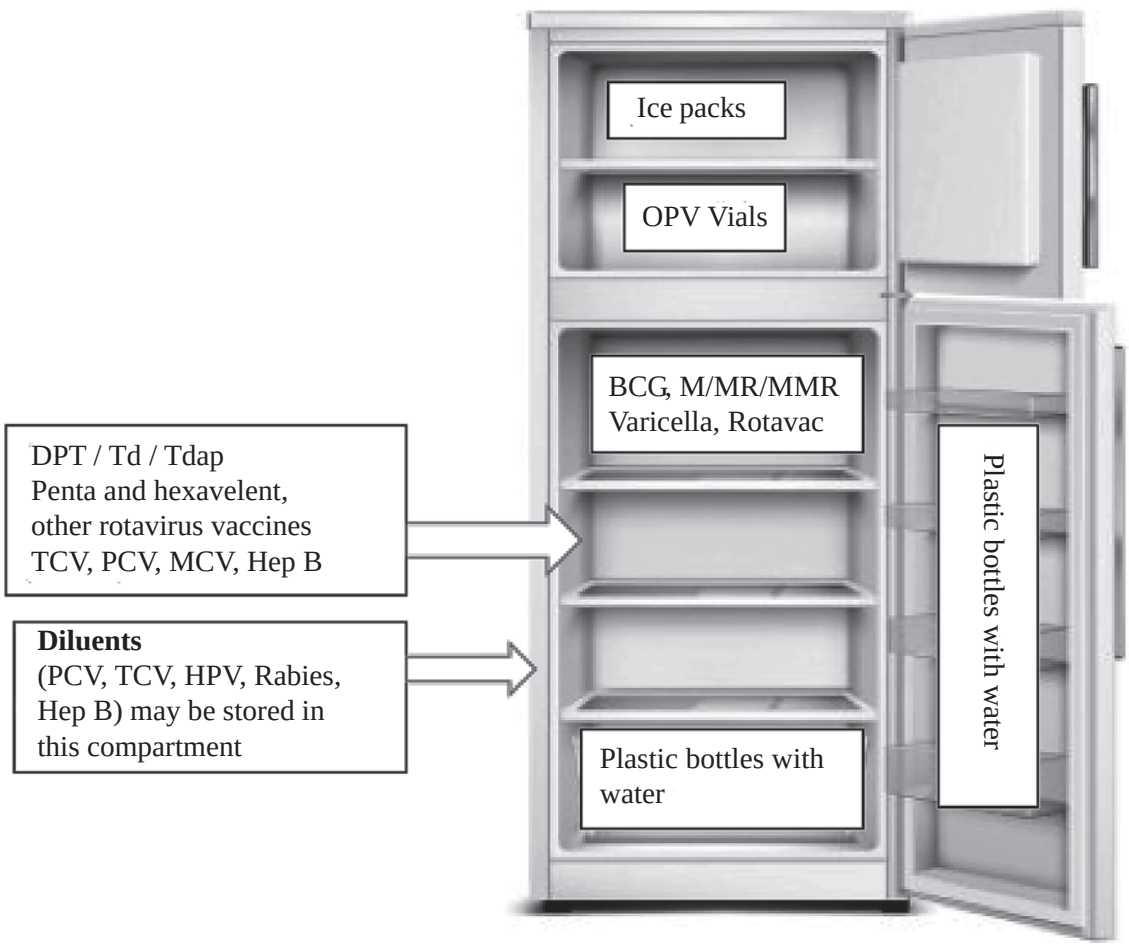

Fig.4. Recommended areas of storage of vaccine and water bottles in the refrigerator

higher in the lower shelves. Vaccines should not be stored in the vegetable compartment or in the door. The recommended areas of storage of the vaccines are given below (Fig.4).

Top compartment (below chiller tray): The most freeze resistant vaccines should be stored in the top compartment. Measles, MR, MMR, BCG, OPV, yellow fever (YF), live-JE, varicella, 116E RVV (Rotavac), live hepatitis A vaccine.

Middle compartment: All the pertussis containing combination vaccines, other rotavirus vaccines, inactivated hepatitis A vaccines, Hep B, HPV, Rabies, pneumococcal vaccine (PCV), Typhoid conjugate vaccine (TCV).

Lower compartment: Diluents, Hep B, HPV, Rabies, PCV, TCV may be stored in this compartment. No vaccine should be stored in the chiller tray except OPV. The freezer should be used for storing ice-packs, to increase the cold mass (Fig.5).

Water bottles with coloured water (to indicate that it is not for drinking purpose) should be stored in the vegetable compartment, door and all unused space, to increase the cold mass.

Vaccines should be stored in labelled trays, with a space of 4 cms between trays to permit cool air circulation between trays (Fig.6). Overstocking the refrigerator should be avoided as it impedes cold air circulation and reduces the likelihood of achieving consistent, stable temperatures throughout the refrigerator.

Vaccines with the shortest expiry dates are to be stored in front. Fresh stocks should be kept at the back and the older stocks moved to the front, the FIFO (First In First Out) principle.

The fridge should be used for storing vaccines only and nothing else.

Regular maintenance of refrigerators

To ensure continued, proper functioning of the refrigerator, the refrigerator coils should be kept dust-free and the drain hole and drip pan meant to remove condensation, should be kept clean and unobstructed. There should be no water or coolant leaks.

The door seals should be checked for leakages. When the fridge door is closed on a sheet of paper, it should not be possible to pull out the sheet easily. If it can be pulled out easily, the seal is not effective and should be replaced. If the door seal is in good shape, it should be cleaned with warm soapy water and then dried.

Ice-lined refrigerators (ILRs)

An ILR is a cabinet with coils all around the interior walls, wherein the water freezes and maintains the requisite range of temperature without constant supply of electricity (Fig.7). An ILR can maintain a temperature from +2°C to +8°C with as little as 8 hours of power supply in 24 hours. Generally, ILRs have a top-opening lid which prevents loss of cold air during door opening. It has inbuilt thermometers and alarm for temperature excursions beyond the safe range.

Holdover time is the duration of time that the ILR can maintain temperature between +2°C and +8°C, in the

Fig.5. Ice Packs in freezer compartment

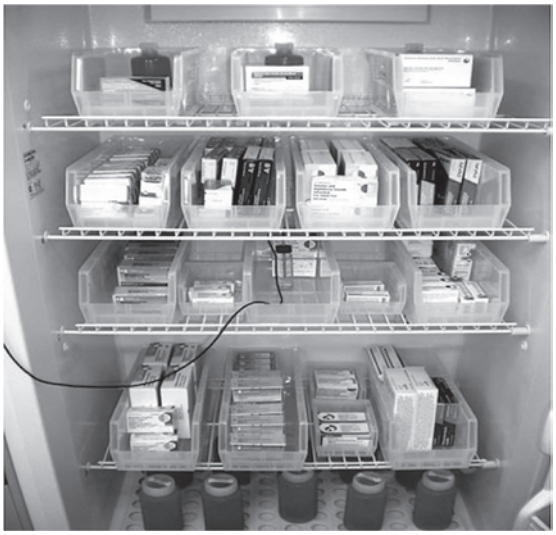

Fig.6.Vaccine stored in trays with adequate spacing in refrigerator

Fig.7. Ice-lined refrigerator

absence of any power supply. The holdover time is at least 24 hours and can extend up to 72 hours. The holdover time of ILR depends on the following factors:

- Ambient temperature - more the ambient temperature, less will be the holdover time;
- Frequency of opening of lid and use of basket
- Quantity of vaccines kept inside and the adequacy of space between the containers (equipment empty/ loaded).

Hence, this is the ideal storage device especially in areas with unreliable power supply. In an ILR, vaccines should be stored in baskets to avoid direct contact with the sides and the bottom, which may result in freezing of vaccines. Since the bottom of the ILR is its coldest part, the most heat sensitive vaccines should be stored at the bottom and the most heat resistant vaccines in the top compartment. This is reverse of the domestic refrigerator.

Measles, MR, MMR, BCG, OPV, YF, live-JE, Varicella, 116E-RV, should be stored in the bottom trays. All the pertussis containing combination vaccines, other rotavirus vaccines, inactivated hepatitis A vaccines, Hep B, HPV, Rabies, PCV, TCV and the diluents, should be stored in the top trays. Since the ILR does not have a freezer compartment, ice-packs cannot be prepared in an ILR.

Fig.8. Purpose-built refrigerator

Purpose-built refrigerators

This is the ideal vaccine storage equipment in the clinic setting, when an assured source of constant power supply exists (Fig.8)

The advantages of a purpose-built refrigerator include:

1. An efficient and quick temperature regulating mechanism avoids excursions outside the recommended range.
2. A small heating element around the cooling coils prevents any frost formation constantly. This prevents temperature excursions often seen with regular defrosting.
3. The temperature in the entire storage area is uniform.
4. Internal temperature is not affected by ambient temperatures.
5. The unit is equipped with inbuilt digital temperature monitoring (inbuilt data logger) and/or digital temperature indicators (minimum and maximum temperature displays)

The only drawback is that the large glass doors do not provide efficient insulation. In the event of a power outage, there is a rapid rise in the cabinet temperature.

Storage of diluents

Diluents should be stored between +2^{o}C and +8^{o} C. If limitations of storage space exist, the diluents may be stored at room temperature but transferred to the refrigerator/ILR at least 24 hours before usage. Using a diluent stored at room temperature may cause a “thermal shock” and inactivation of the vaccine.

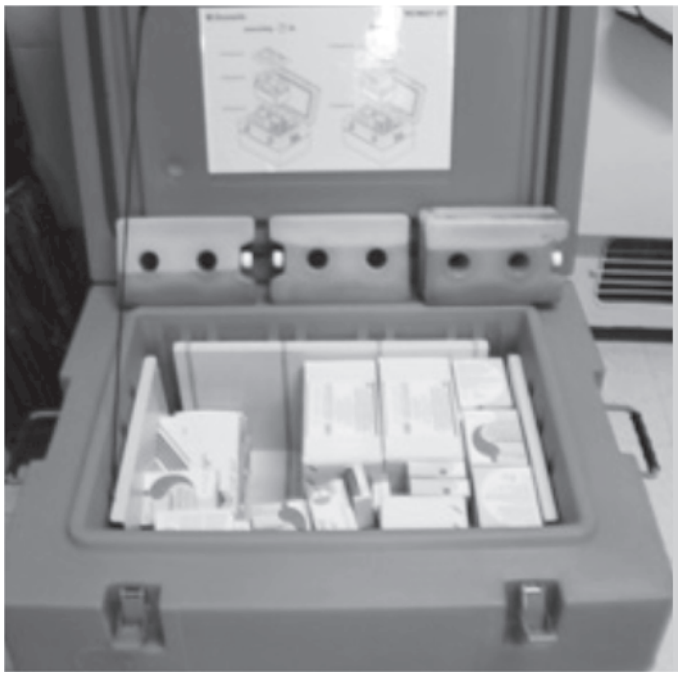

Fig.9. Cold boxes with conditioned ice packs

Cold boxes

A cold box is an insulated container that can be lined with ice-packs to keep vaccines and diluents cold during transportation and/or short period storage (from two to seven days) (Fig.9).[8]

Cold boxes are to be packed with the recommended number of conditioned ice packs on all sides. Freeze sensitive vaccines should not come in contact with the ice pack. This can be assured by packing them in plastic covers.

Cold boxes vary in size, storage capacities and the number of icepacks necessary to maintain the recommended temperature range.

Fig.10. Vaccine carrier with icepacks

Cold boxes are used to collect and transport monthly vaccine supplies from district stores to the health facility, store vaccines when the refrigerator is out of order or being defrosted and for outreach and mobile sessions in addition to vaccine carriers. Cold boxes can maintain the recommended temperature range for 5 days.

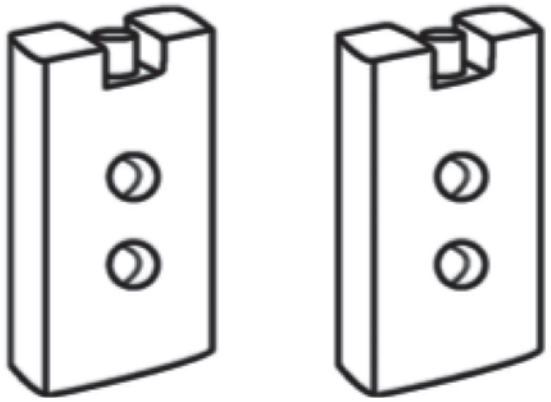

Fig.11.Ice packs

Vaccine carriers

These are smaller cold boxes which are used to carry a day's supply of vaccines for outreach activities (Fig.10).[8] Generally, 4 icepacks are necessary for vaccine carriers. Unopened, they can maintain the recommended temperature range, for 48 hours.

Ice-packs

Ice packs are a crucial component of the cold chain. These are flat, plastic, waterproof and leak-free boxes of standard sizes. These packs are filled with tap water till the mark, tightly closed and kept in the deep freezer, till the water is frozen. Frozen icepacks are at a temperature of -20^{o}C and cannot be used as such (Fig.11). They need to

Fig.12. Foam pads

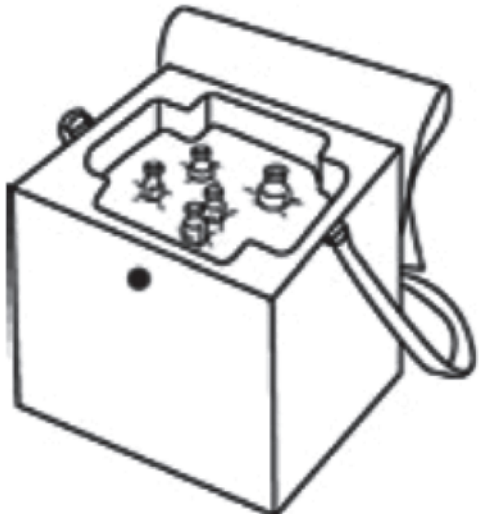

Fig.13. Foam pads with slits in position

undergo "sweating". When frozen icepacks are kept at room temperature, the ice partially melts, with drops of water occurring on the surface. These icepacks have an initial temperature of 0°C.

Cool water packs contain liquid water at an initial temperature of +5°C or less. These are used with the more freeze sensitive vaccines. The icepacks used for vaccine carriers are different in size from those used for cold boxes.

Foam pads

This is a piece of soft sponge- like material that fits snuggly on top of the ice packs inside a vaccine carrier while still permitting the lid of the vaccine and is supplied by the manufacturer of the vaccine carriers[8] (Fig.12). The foam pads have slits in which the vaccine vials can sit snuggly during immunization sessions (Fig.13). The foam pad serves to keep the interior of the vaccine carrier cool in spite of the lid being open. Even with a foam pad, however, it is important to keep the hard vaccine carrier lid closed whenever possible to conserve the inner temperature.

Temperature monitoring devices

Vaccine Vial Monitor (VVM)

This is a time-temperature indicator which contains diacetylene, a heat-sensitive, compound, which is colorless, but on exposure to changing temperatures, undergoes serial color changes which depends on the temperature and duration of exposure to that temperature (Fig.14).[7] The VVM records the cumulative heat exposure through a gradual change in color. If the color of the inner square is the same color or darker than the outer circle, the vaccine has been exposed to too much heat and should be discarded. VVMs are designed to meet the vaccine's heat stability curve, allowing a margin of safety. Good correlation has been observed between the vaccine vial monitor and vaccine potency of OPV.

VVMs are based on the accelerated degradation test (ADT), rather than by estimating loss of potency during long periods of storage at different temperatures (Table I). It is to be noted that only WHO pre-qualified vaccines are accompanied by a VVM.

The position of the VVM on the vaccine vial/ampoule is of significance.[8] (Fig.15 a and b). When the VVM is attached on the label, the vaccine vial once opened can be used for next 28 days (liquid or freeze-dried). When the VVM is attached anywhere other than label (cap or neck of ampoule), the vaccine vial, once opened, must be discarded after immunization session or within six hours of opening, whichever comes first.

Integrated digital thermometers

These temperature measuring devices are inbuilt in most WHO prequalified cold storage equipment.[7] (Fig.16). A sensor is placed in the middle compartment, away from side walls and other containers, which constantly records the temperature and displays it on a unit attached to the wall of the refrigerator. Some are also equipped with an

Table I. Different types of VVM

VVM Type	No. days to end point at +37°C	No. days to end point at +25°C	Time to end point at +5°C	Vaccines
VVM 30	30	193	>4 years	BCG, HPV, HBV, Rabies, PCV
VVM 14	14	90	>3 years	DPT/DT/TT, YF, MV/MR/MMR, Liquid pentavalent vaccine (LPV), IPV
VVM 7	7	45	>2 years	IPV
VVM 2	2	NA	225 days	OPV, 116E Rotavirus vaccine

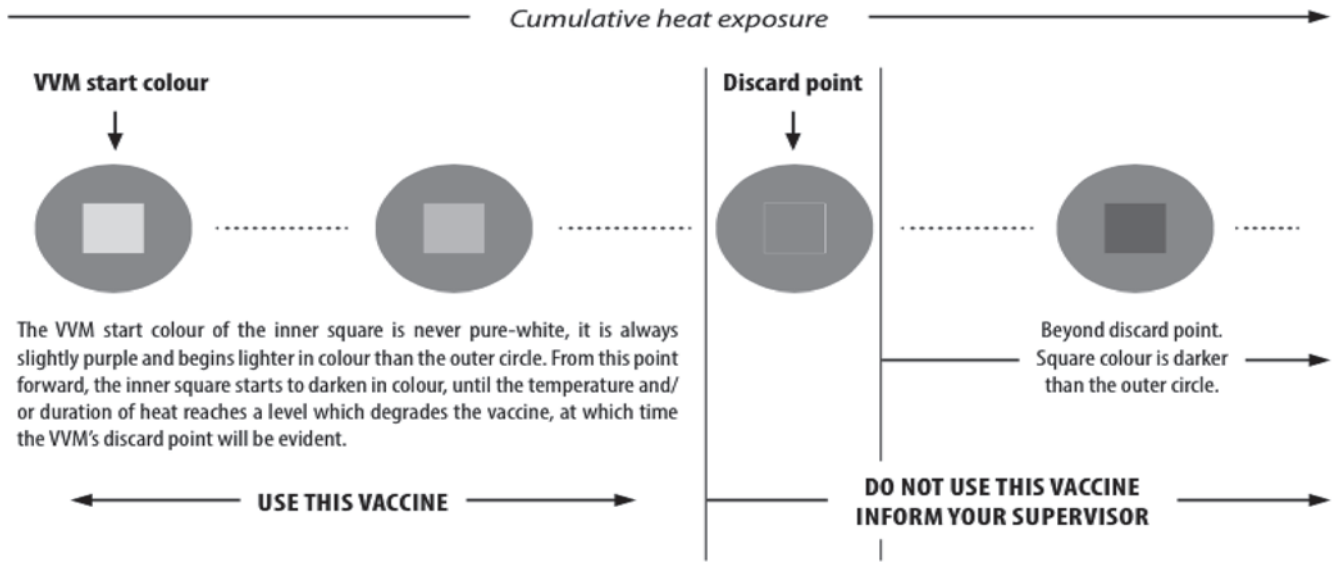

Fig.14. VVM showing colour changes sequences and interpretation

inbuilt alarm to alert temperature excursions outside the recommended range. These devices need 6 monthly calibration and change of batteries.

Digital data loggers (DDL)

A DDL provides the most accurate storage unit temperature information[7] (Fig.17). It measures the temperature at preset intervals, has alarms to warn of temperature excursions outside the recommended temperature range and store this information. Temperature data from a DDL can either be downloaded to a computer using special software or retrieved from a website.

Digital Maximum-Minimum Thermometers (Fig.18)

This device monitors the temperature and display 3 values: current temperature, maximum temperature

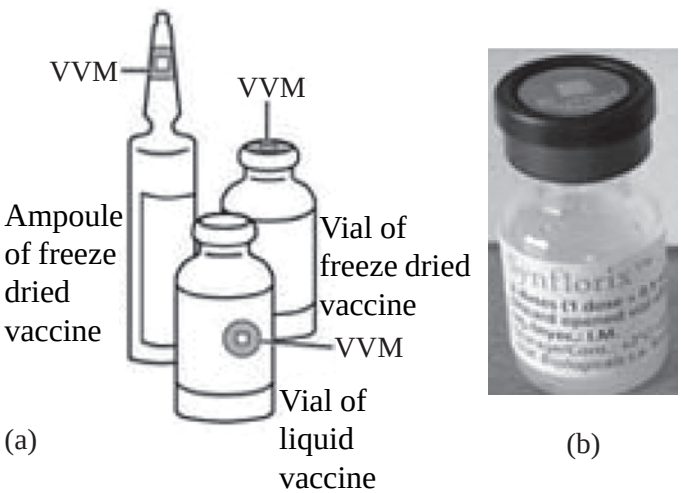

Fig.15. Position of the VVM

Fig.16. Digital thermometer

recorded since last reset and minimum temperature recorded since last reset. However, storage and retrieval of data is not possible.

Maintenance of data loggers[6]

- Recalibrate the data logger annually.
- Change the data logger battery at least every 12 months or as indicated by the manufacturer.

Fig.17. Digital data logger

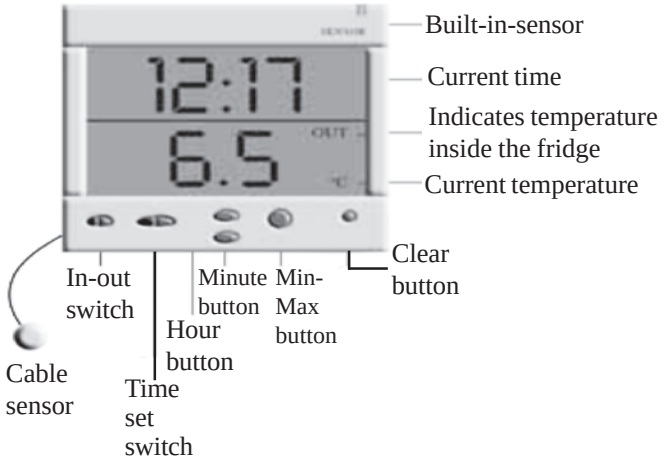

Fig.18. Digital Maximum-Minimum Thermometers

Fig.19. Electronic freeze indicators

- Check the accuracy of the thermometer at least annually.
- Change the thermometer battery at least every 12 months or as indicated by the manufacturer.

Maintenance of thermometers[6]

Thermometers require annual checks to ensure accurate measurement. This can be done in a simple way. Take a plastic cup, fill 2/3rd with water and keep in freezer till a thin layer of ice is formed. This may take about 2-2.5 hours.

Place the thermometer probe in the cup such that it should not be touching the side or bottom of the cup. After 2 minutes, record the temperature. It should read between + 1^0 C and – 1^0 C. If the recording is outside this range, change the batteries and repeat the process. If the recording is still beyond this range, change the thermometer.

Electronic freeze indicators : May be used as a backup indicator for potential freeze exposure in low temperature locations within a cold room (Fig.19). These are for single use and once the alarm has been activated, the device cannot be used again.

Devices which are not recommended should not be used for monitoring the cold chain. The refrigerator temperature should be recorded twice daily, at 10 am and 4 pm and a log maintained in a tabular form (Table II).

Voltage stabilizer

A voltage stabilizer is an electronic equipment that ensures a constant output voltage of 220 volts whatever be the variation in input voltage and thus safeguards equipment

Table II. Refrigerator temperature chart

Month ..... Year .....

Date	Temperature (^{0}C)		Power Failure (in hours)	Remarks (if any)
	10 AM	4 PM		
1.				
2.				
3.				
4.				
5.				
6.				

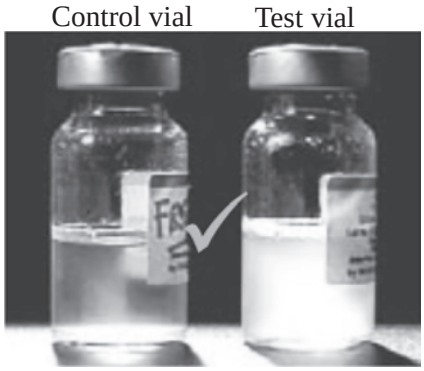

Fig.20a. Shake test passed Vaccine usable

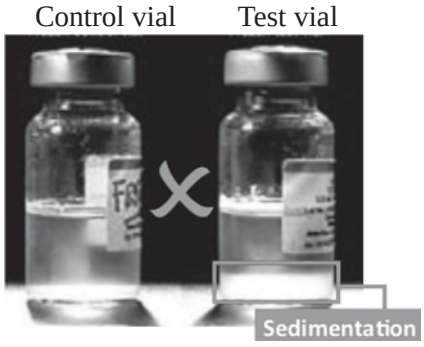

Fig.20b. Shake test failed – Do not use vaccine

from excessive voltage variation. The WHO has strongly recommended the use of voltage stabilizers in all cold chain equipment.

Back-up power supply

This is recommended at all levels of vaccine storage.

Shake test - This is a validated test which assesses the usability of a frozen vaccine, when the freeze indicator is activated.[4]

Method: The suspected frozen vial is the test sample. Control sample is a vaccine vial of the same antigen, same manufacturer, and same batch number as the suspect vaccine vial.

a) The control should be frozen solid by keeping overnight in the freezer.

b) It should be thawed by keeping at room temperature.

c) The control and the test vials should be held together between thumb and forefinger, and vigorously shaken for 10-15 seconds.

d) Both vials are placed on a flat surface, side-by-side and observed for 30 minutes.

e) The rate of sedimentation is compared.

f) If the sedimentation rate in the 'Test vial" is slower than in the "Frozen vial", the vaccine has not been damaged, it has passed the shake test. It can be used.

g) If the sedimentation rate is similar in both vials or if sedimentation is faster in the "Test" vial than in the "Frozen" vial, the vaccine is damaged, has failed the shake test and should not be used (Fig.20a and b).

Multi-dose vaccine policy 2014 (MDVP)

According to this policy, WHO pre-qualified, multi-dose vials of vaccines, can be kept and used for up to 28 days after opening, provided the following prerequisites are met[9] :

1. The vaccine is approved for use for up to 28 days after opening the vial, as determined by WHO
2. The expiry date of the vaccine has not passed.
3. The vaccine vial has been and will continue to be stored at WHO / manufacturer-recommended temperatures; and the VVM, if attached, has not reached the discard point and the vaccine has not been damaged by freezing.
4. Due precautions have been taken while drawing out doses from the vaccine vial.

If all of the criteria cited above are present, the vaccine vial may be kept and used for up to 28 days after opening, or until all the doses are administered. DPT, TT, Hep B, OPV, PCV, Hib containing pentavalent vaccine (Penta) and injectable inactivated poliovirus vaccine (IPV) come under the MDVP (also known as 'Open vial policy').

Dealing with a cold chain breach

A 'cold chain breach' has occurred if vaccine storage temperatures have been outside the recommended range of +2°C to +8°C[6]. It excludes fluctuations upto +12°C, lasting no longer than 15 minutes, as may occur when stock taking or restocking refrigerators. This may occur during power outages or inadvertent switching off of power source. The latter can be prevented by using power outlets without switches or taping the switch to render it inoperable.

During power outages of short duration less than 4 hours, the refrigerator with ice packs, if unopened, can maintain temperature within the safe range. However the refrigerator temperature should be checked periodically and if it rises beyond + 8°C, the vaccines should be kept in a fully prepared cold box or shifted to a site with adequate, functioning cold storage facilities. When the power supply is restored, the vaccines should be shifted to the refrigerator only after the temperature stabilizes at +2°C to +8°C.

An action plan should be ready and rehearsed for such eventualities.

Advances in cold chain systems

In the "Controlled Temperature Chain" (CTC), vaccines are kept at temperatures outside of the recommended cold chain of +2°C to +8°C for a limited period of time under monitored and controlled conditions.[10] In a CTC, the vaccine vial can be exposed just once, to ambient temperatures not more than +40°C and for the specified number of days, just before administration. Only 4 vaccines are recommended for use in CTC. These are the HPV vaccine, oral cholera vaccine (OCV), tetanus toxoid vaccine (including TT, Td, or other TT-CVs [tetanus-toxoid-containing vaccines]) and hepatitis B vaccine birth dose (HepB-BD).

Electronic Vaccine Intelligence Network (eVIN) is a smartphone application, which digitally maintains vaccine stocks and monitors the temperature of the cold chain.[11] eVIN provides real-time information on vaccine stocks and flows, and storage temperatures across all cold chain points. It is now operative in 32 states and union territories with 23057 cold chain points in 585 districts and 23900 electronic data loggers.

Innovations in better insulation technologies, vacuum panels and high thermal resistance gels, may enable use of cold boxes up to a month.[12]

User- independent freeze prevention (UIFP), which ensure vaccine freeze prevention, without any intervention required by the user, are being actively investigated to prevent freeze damage to vaccines, during storage.[13] Currently, there are 23 pre-qualified Solar Direct Drive (SDD) and mains-powered refrigerators that provide Grade A freeze prevention.

Use of phase change materials (PCM) in cold chain equipment is being investigated.[14] This will enable strict maintenance of the recommended temperature range in the cold chain system.

Points to Remember

- ***Cold chain is a system of storing and transporting vaccine at the recommended temperature range from the point of manufacture to point of use.***
- ***The main components of the cold chain are personnel, equipment and protocols.***
- ***The cold chain equipment used for storing and transporting vaccines may be active or passive systems. Active system refrigerators operate on electricity obtained from a power grid and off-grid using either LPG, kerosene or solar power. Passive systems consist of cold boxes and vaccine carriers, involving no active refrigeration mechanism.***
- ***Vaccine storage in the refrigerators should be based on thermolability of the vaccines and adequate knowledge of temperature zones within the device.***
- ***Temperature monitoring devices include the vaccine vial monitors, thermometers, data loggers and freeze indicators.***
- ***A 'cold chain breach' is said to have occurred if vaccine storage temperatures are beyond the recommended range of +2°C to +8°C and an action plan should be made for such eventualities.***

References

1. WHO. Immunization supply chain and logistics. Available at https://www.who.int/immunization programmes_ systems/supply_chain/en/. Accessed on 28 September, 2020.
2. Centre for Disease Control.Vaccine Storage and Handling Tool kit. https://www.cdc.gov/vaccines/hcp/admin/storage/toolkit/index.html. Accessed on 28 September, 2020.
3. Galazka A, Milstien J, Zaffran M. Thermostability of vaccines. WHO/GPV/98.07. Available at http://www.who.ch/gpv-documents/. Accessed on 28 September, 2020.
4. WHO. Immunization in practice module 2: the vaccine cold chain. Geneva: WHO; 2014. Document no. WHO/IVB/04.06. Available from: http://www.who. int/immunization/documents/iip2014mod2aug4.docx. Accessed on 30 September, 2020.
5. Inclen trust. In-depth analysis of cold chain, vaccine supply and logistics management for routine immunization in three Indian states: An INCLEN program evaluation network study. Available at http://inclentrust.org/inclen/wp-content/uploads/Cold-Chain-Full-Report.pdf. Accessed on 2 October, 2020.
6. Australian Government. Dept of Health.. National vaccine storage guidelines. Strive for 5. 3rd edn. Available at https://www.health.gov.au/sites/default/files/documents/2020/04/national-vaccine-storage-guidelines-strive-for-5.pdf. Accessed on 30 September, 2020.
7. Shastri D. Vaccine storage and handling. In: Advisory Committee on Vaccines and Immunization Practices, Indian Academy of Pediatrics. IAP Guidebook on Immunization 2018-2019. Balasubramanian S, Shashtri DD, Shah AK, Pallab Chatterjee, Harish Pemde, Shivananda S, Vijaya Kumar Guduru (eds), 3rd Edn, Jaypee Brothers Medical Publishers, New Delhi, 2020; pp50-68.

8. WHO. Module 3. The cold chain. WHO. Available at https://apps.who.int/iris/bitstream/handle/10665/64982/WHO_EPI_TRAM_98.03.pdf?sequence=3. Accessed on 2 October, 2020.
9. Ministry of Health & Family Welfare, Government of India. Handbook for vaccine & cold chain handlers. New Delhi, 2016, UNICEF, WHO. Accessed from: https://www.technet-21.org/media/com_resources/trl/2244/multi_upload/Unicef_Cold%20Chain%20Handlers%202016.pdf.
10. World health organization. Controlled temperature chain. Available at https://www.who.int/immunization/programmes_systems/supply_chain/ctc/en/. Accessed on 10 October, 2020.
11. Vikaspedia.. eVIN project. Available at https://vikaspedia.in/health/health-care-innovations/health-system-strengthening-1/evin-project-of-health-ministry. Accessed on 10 October, 2020.
12. Quinn FJ. New cold chain solutions meet regulatory, shipper requirements. Pharm Commerce 2014; 9(2): 24-25.
13. WHO. PQS target product profile: enhanced mains-powered refrigerators or combined mains-powered refrigerator and vaccine freezer. Geneva: WHO; 2015. Report no. WHO/PQS/E003/TPP 04.1. Available from: http://apps.who.int/immunization_standards/vaccine_quality/pqs_catalogue/catdocumentation. aspx?id_cat=17. Accessed on 10/10/2020.
14. PATH. Investigation of Phase-Change Materials for Vaccine Cold Chain Equipment. Seattle: PATH; 2016. Available from https://path.azureedge.net/media/documents/DT_pcm_summary_rpt1.pdf. Accessed on 10 October, 2020.

CLIPPINGS

Epidural Analgesia During Labor and Risk of Autism Spectrum Disorders in Offspring

Although the safety of labor epidural analgesia (LEA) for neonates has been well documented, the long-term health effects of LEA on offspring remain to be investigated.

Data were derived from a large birth cohort from an integrated health care system (Kaiser Permanente Southern California - KPSC) with standard clinical practices and comprehensive electronic medical records where the continuum of prenatal, perinatal and postnatal care for both mother and baby are standardized.

This retrospective longitudinal cohort study included singleton children born by vaginal delivery at 28 to 44 weeks' gestation in KPSC hospitals between January 1, 2008 and December 31, 2015. Per KPSC guidelines, a brief screening checklist (a modified version of the Checklist for Autism in Toddlers) was administered to all children between the ages of 18 and 24 months to screen for developmental delays, including autistic spectrum disorders (ASD). A clinical diagnosis of ASD was based on pediatric developmental specialist evaluations.

The exposure variable was LEA administered during labor and delivery. The outcome measure was the presence or absence of ASD during the follow-up period, which was identified by International Classification of Diseases, Ninth Revision codes 299x or equivalent KPSC codes (autistic disorders, Asperger syndrome, or pervasive developmental disorder not otherwise specified and excluded childhood disintegrative disorder and Rett syndrome). Codes from at least 2 separate visits were required for an ASD diagnosis and were validated with a positive predictive value of 88%.

In this multiethnic population-based clinical birth cohort that included 147 895 children, ASD were diagnosed in 1.9% of the children delivered vaginally with epidural analgesia vs 1.3% of the children delivered vaginally without the exposure, a 37% relative increase in risk that was significant after adjusting for potential confounders.

This study suggests that exposure to epidural analgesia for vaginal delivery may be associated with increased risk of autism in children; further research is warranted to confirm the study findings and understand the potential mechanisms.

Qiu C, Lin JC, Shi JM, Chow T, Desai VN, Nguyen VT, et al. Association Between Epidural Analgesia During Labor and Riskof Autism Spectrum Disorders in Offspring. JAMA Pediatr. 2020;174(12):1168-1175. doi:10.1001/jamapediatrics.2020.3231Published online October 12, 2020.

VACCINOLOGY I

OVERVIEW OF VPD SURVEILLANCE IN INDIA

*** Bhaskar Shenoy**

Abstract: *Disease surveillance is an important component of public health programs. Vaccine preventable disease surveillance consists of collection of data on vaccine preventable diseases that is utilized for focused interventions for control, elimination or eradication of the disease under surveillance. The key objectives of efficient surveillance system are to assess the burden of a disease in the community, monitor the progress of interventions for disease reduction, assess the impact on disease epidemiology and early detection of outbreaks to implement appropriate control measures. In India, the main vaccine preventable diseases under surveillance are polio, measles, rubella, diphtheria, pertussis and neonatal tetanus. All health facilities including government, NGOs, private clinics, hospitals and laboratories should notify all cases under surveillance including tuberculosis to local health authorities every month. Government of India implements all these programs in coordination with World Health Organization and other partners in immunization.*

Keywords: *Vaccine preventable diseases, Surveillance, Children.*

Public health surveillance is the continuous and systematic collection, analysis and interpretation of health-related data needed for the planning, implementation and evaluation of public health practice.[1] Vaccine preventable diseases (VPD) surveillance refers to the application of this collective process to estimate the incidence and prevalence of VPDs and to utilize the generated data for focused actions and interventions aiming at either control/ elimination/eradication of the diseases concerned. This is vitally important for its potential to provide information to policymakers, monitor immunization programs including vaccine introduction, coverage and to formulate outbreak response strategies.

* Head, Department of Pediatrics and Chief, Division of Pediatric Infectious Diseases, Manipal Hospitals, Bangalore.

email: bshenoy@gmail.com

VPD surveillance also helps in detecting changes in the epidemiology of VPDs over time due to vaccine use and other preventive measures. As the burden of a VPD is reduced, the objectives and design of the surveillance system may be modified accordingly.

In India, surveillance exists for many VPDs. A robust acute flaccid paralysis (AFP) surveillance system in place for many years led India to achieve polio elimination. A similar strategy has been utilized as a backbone to immunization related surveillance for other diseases. The term "VPD surveillance" refers to surveillance for diphtheria, pertussis and neonatal tetanus (NNT). For measles (M) and rubella (R), there is 'modified case based MR surveillance' and surveillance for tuberculosis (TB) is under 'National TB elimination programme (NTEP)' - previously Revised National Tuberculosis Control Programme. Government of India (GOI) implements all these programs in coordination with World health organization (WHO) and other organizations involved in immunization. The criteria for prioritizing surveillance of communicable diseases including VPDs is given in Box 1.

Objectives of vaccine preventable disease surveillance[2]

- To detect areas with VPDs and trend of disease over time
- Monitoring disease elimination and eradication efforts
- Detection of outbreaks and new pathogens
- Evidence for new vaccine introduction
- Evaluation of immunization programme performance
- Vaccine effectiveness, impact on disease burden
- Changes in pathogen strains or types

Types of vaccine-preventable disease surveillance[3,4]

Outbreak-based surveillance: Focuses on detection of outbreaks - occurrence of disease cases in excess of normal expectancy. Few reported cases are investigated to confirm or rule out outbreaks of diseases (measles/rubella).

Case-based surveillance: Focuses on each and every case (AFP, diphtheria, pertussis and NNT surveillance).

Box 1. Criteria for prioritizing communicable disease surveillance, including VPDs

- Disease burden and endemicity (natural level of disease occurrence)
- Severity and case fatality ratio
- Epidemic potential
- Potential for emergence of virulence or changing pattern of disease
- Prevention and control and elimination potential
- Social and economic impact
- International reporting regulations, such as International Health Regulations
- Public perception of risk

Sentinel surveillance: Refers to a system that captures cases at one or more specialized sites, such as hospitals, clinics or pharmacies. Sentinel surveillance can provide useful information about the epidemiology, risk factors and pathogens causing disease such as circulating strains and the impact of immunization. This system provides substantial data over the years and provides information to signal trends and monitors the burden of disease in a community (for example, congenital rubella syndrome surveillance). Sentinel surveillance is conducted only in selected locations and thus it may not be effective for detecting rare diseases or diseases which occurs outside the catchment areas of the sentinel sites. E.g Indian national rotavirus surveillance network, Hib surveillance.

Nationwide or sub national surveillance: VPD surveillance can be nationwide or intentionally limited to a defined part of the geographical area and later widened to cover the entire country in a phased manner. Sub national surveillance might be considered if the VPD burden is confined to certain regions of the country, or if there is greater capacity to conduct high-quality surveillance in a particular geographic area. However, the cases captured through sub national surveillance might not be representative of those occurring throughout the entire country. For VPDs with control goals, sub-national surveillance may be acceptable for determining risk factors or evaluating the impact of a vaccine. For VPDs targeted for elimination or eradication, nationwide surveillance that strives to detect all cases is essential. Eg. measles, polio and diphtheria.

Facility-based and community-based surveillance: Preselected health facilities network report cases approaching them in facility-based surveillance system whereas community based surveillance involves active case searches in community. Both the systems are complementary to each other. Community-based surveillance can potentially detect less severe VPD illness or diseases not normally captured at health facilities because they are considered a normal part of childhood (such as measles) or have an associated stigma (such as death from neonatal tetanus). For polio eradication, a community-based surveillance strategy is critical in order to detect all cases, regardless of severity and break the chains of transmission.

Active/Passive surveillance: Active surveillance involves active searching for cases (in case of diseases targeted for eradication/elimination) in health facilities and in community. e.g., weekly household visit to enquire about fever with rash by health worker. Passive surveillance involves reporting from health facility. In active surveillance, designated surveillance staff is directly involved in detecting cases. For example, public health surveillance staff may do a regular review of facility registers and have regular contact with clinicians regarding potentially missed cases (e.g. AFP surveillance by surveillance team). Compared to passive surveillance, active surveillance is more resource intensive and expensive. It is often used for VPDs in the elimination or eradication phase.

Aggregate versus case based: In aggregate surveillance, only the number of cases is collected and reported from clinic admission registers, logbooks or other records. No individual level data is collected, but cases may be aggregated by subgroups such as age, sex or locality. In contrast, case based surveillance requires the collection and reporting of detailed information on each case of a VPD. Examples of such details include age, sex, residence, vaccination status and risk factors.

Population based: Population based surveillance attempts to capture all cases in a well-defined catchment population (e.g. the entire population of a country). When the catchment population is defined, one can use the total population number as the denominator to calculate VPD incidence. This makes it possible to understand the impact of a vaccine on disease burden over time and compare VPD incidence across countries or regions.

Clinical versus laboratory based surveillance: Laboratory supported surveillance is a critical component of strategies to control, eliminate and eradicate infectious diseases including VPDs.[5] The difference between clinical and lab based surveillance is dependent on whether the case detection is initiated based on clinical diagnosis or laboratory test results. Many surveillance systems start with

a clinical definition to capture suspect cases (for example, a syndrome like diarrhea or fever-rash) and then use laboratory tests to confirm cases. In laboratory-based surveillance, a laboratory result confirming a VPD case is the starting point for inclusion in surveillance.[6,7] Laboratories or hospitals report these laboratory-confirmed cases to public health authorities, either as part of national disease reporting requirements or sentinel surveillance networks. Eg. measles, polio, diphtheria, meningococcus and rubella.

Syndromic surveillance platforms for VPD

A particular type of integrated surveillance approach is syndromic surveillance. This term refers to the use of a clinical syndrome-a constellation of symptoms and signs, as the case definition for detection of suspect cases of a VPD. Using syndromic surveillance platforms for multiple VPDs can be more efficient than doing surveillance for a single disease (Table I). For example, acute flaccid paralysis (AFP) surveillance for polio is a type of syndromic surveillance that captures not just polio but also Guillain-Barre syndrome or other conditions that cause flaccid paralysis. Surveillance of fever-rash syndrome is used for measles and rubella but could also include dengue, parvovirus B19 or other viral causes.[8] Laboratory testing for multiple pathogens is done according to a predefined algorithm to determine the final diagnosis and assign the cases to the correct VPD. Syndromic surveillance can be used for initial case detection, but laboratory confirmation should occur to increase the accuracy of the system.

COVID-19 Surveillance

The aim of national surveillance for COVID-19 is to enable public health authorities to reduce transmission of COVID-19, thereby limiting associated morbidity and mortality. The objectives of COVID-19 surveillance are to enable rapid detection, isolation, testing and management of cases and to monitor trends in COVID-19 deaths and quarantine of contacts. It is critical that new cases and clusters of COVID-19 be detected rapidly before outbreaks and/or widespread transmission occurs.

Surveillance approaches for COVID-19

Surveillance in the Community: Community-based surveillance (CBS) is the systematic detection and reporting of events of public health significance within a community by community members - may serve to bridge the gap between the community and the health system.

Hospital-based surveillance: Patients with probable or confirmed COVID-19 admitted to hospitals should be notified to national public health authorities within 24 hours of identification.

Sentinel site (ILI/ARI/SARI) surveillance:The advantage of utilizing a sentinel surveillance system is that a systematic, standardized approach to testing is used and not affected by changes in testing strategies affecting the other COVID-19 surveillance approaches.

Mortality Surveillance: The number of COVID-19 deaths occurring in hospitals should be reported daily.

Event-based surveillance (EBS): EBS captures unstructured information from formal and informal channels such as online content, radio broadcasts and print media across all relevant sectors to complement conventional public health surveillance efforts. Telephone hotlines made available to the public for advice and referral to health-care services may provide an early indication of disease spread in a community.

Participatory surveillance: Participatory disease surveillance enables members of the public to self-report signs or symptoms, without laboratory testing or assessment by a health care provider

Serological surveillance: Population-based surveys of antibody seropositivity and the use of serology in specific settings/populations can help to provide estimates of the proportion of a population that has been infected by SARS-CoV-2 virus as measured by antibodies. This can assess the extent of infection in the general or subpopulations, in specific age groups and potentially, the proportion of unrecognized infections (e.g., asymptomatic or subclinical infections.[9]

Various steps of VPD surveillance

Successful VPD surveillance requires alignment with a country's objectives, meticulous planning and ongoing attention to the daily operations at each step of surveillance. Surveillance infrastructure, including the reporting network and laboratory capacity must first be established. For ongoing surveillance, the routine steps are given in Box 2.

Box 2. Steps in VPD surveillance

- Case detection
- Case investigation
- Sample collection and laboratory testing
- Case classification
- Data analysis and interpretation
- Reporting of data
- Feedback

Table I. Syndromic surveillance platforms for VPDs

Clinical syndromes	VPDs associated with clinical syndromes	Non-VPD causes of syndromes
Diarrhea (including watery and bloody)	Rotavirus, Cholera	Shigella* Norovirus*
Acute jaundice	Hepatitis - A,B,E Yellow fever	Hepatitis C, Leptospirosis Liver flukes
Fever with rash	Dengue (Once vaccine available) Measles Rubella Varicella Typhoid Meningococcus	Scarlet fever (groupA Streptococcus) *Scrub typhus Erythema infectiosum (Parvovirus B19) Roseola infantum (human herpes virus 6) Enterovirus (echovirus, coxsackievirus) Infectious mononucleosis Kawasaki disease Chikungunya virus Zikavirus
Acute flaccid paralysis (AFP)	Polio Japanese encephalitis Herpes zoster Rabies Tick-borne encephalitis	Enteroviruses (coxsackie virus, echovirus, enterovirus 71) West Nile virus, Guillain-Barré syndrome, traumatic neuritis, transverse myelitis
Meningoencephalitis (ME)/ acute encephalitis syndrome (AES)	Meningococcus, Pneumococcus, Hemophilus influenzae type b (Hib) Japanese encephalitis Tuberculous meningitis Influenzae Dengue	Herpes simplex virus West Nile virus Saint Louis encephalitis virus Scrub typhus Acute disseminated encephalomyelitis (ADEM) Autoimmune encephalitis
Severe acute respiratory illness (SARI)/ Influenza-like illness (ILI)	COVID-19 Influenzae Pertussis Hemophilus influenzae type b (Hib)	Respiratory syncytial virus* Other respiratory infections
Persistent cough	Pertussis	Other respiratory infections (e.g. Mycoplasma pneumoniae) Tuberculosis
Meningitis/ pneumonia/sepsis	Meningococcus, Pneumococcus, Hib, Typhoid, Japanese encephalitis	

*Vaccines not currently available, but in advanced phase of development.

Prioritization is given for VPD surveillance based on the case fatality rate, tendency to cause epidemics, availability of potential targets for elimination or eradication and the commitment to follow international health regulations.

Steps of an outbreak investigation

If a VPD outbreak is identified, an investigation should be conducted by surveillance officers or other public health officials or both. The investigation of outbreaks of disease, including VPDs, is often broken down into the following series of steps:[10,11]

1. Verify the diagnosis and confirm the existence of an outbreak.
2. Establish an outbreak case definition (or modify existing one used in VPD surveillance).
3. Conduct case-finding and data collection.
4. Describe the outbreak.
5. Generate and test hypothesis regarding the source and cause of outbreak (for example, failure to vaccinate versus vaccine failure).
6. Implement control and prevention measures (vaccination for VPDs among other public health interventions).
7. Analyze the results and communicate findings. E.g. Muzaffarpur outbreak of acute encephalitis syndrome (AES) in 2019, was later diagnosed as toxic encephalopathy.
8. Strengthen VPD surveillance and the immunization program and potentially change vaccine policy.

Surveillance system in India

Multiple surveillance systems are operational in India[4]

- National Public Health Surveillance Project (formerly national Polio Surveillance Project-WHO-NPSP): This is an efficient surveillance system, functional since 1997 for polio surveillance. This public private coordination has led to excellent reporting and ultimately elimination of polio from India and many other countries. This system is now modified to include other VPDs. A laboratory supported case-based surveillance system is established for selected VPDs like measles, rubella, diphtheria, pertussis and NNT.
- Integrated Disease Surveillance Project (IDSP): This surveillance system focuses on epidemic prone diseases to detect the early warning signals, so that timely and effective public health actions can be initiated in response to health challenges in the country. A Central Surveillance Unit (CSU) is established at the National Centre for Disease Control (NCDC), formerly National Institute of Communicable Diseases (NICD), Delhi. The State Surveillance Units (SSUs) are established at all State/union territory headquarters and District Surveillance Units (DSUs) at all districts in the country. Data is generated, compiled, analyzed at district level and shared with SSU and CSU.
- Central Bureau of Health Intelligence (CBHI): CBHI is the health intelligence wing of the Directorate General of Health Services (DGHS) in the Ministry of Health and Family Welfare (MoHFW) GOI, with the vision to have "a strong health management information system in entire country". CBHI has a web-based data entry portal for collation of data at the national level. It regularly brings out an annual publication in the form of 'National Health Profile' based on the health data collected from all health directorates of states and union territories."

Infectious disease surveillance project (IDsurv): Indian Academy of Pediatrics (IAP) platform for reporting of infectious diseases.

Indian Academy of Pediatrics (IAP) has developed an infectious disease surveillance and Adverse Events Following Immunization (AEFI) reporting system known as IDsurv.org. IAP members are motivated to provide the required information on this website.[12]

The main objectives of the program are

- To generate data on key VPDs in India.
- To sensitize members about serious AEFI and generate data.

The key infections targeted under this project are: Acute bacterial meningitis, chickenpox, diphtheria, dengue and hepatitis

Vaccine-preventable disease surveillance in India

Laboratory supported case-based VPD surveillance is launched by GOI in a phased manner. It includes case-based surveillance of measles, rubella, diphtheria, pertussis and NNT in addition to AFP and adverse event following immunization(AEFI) surveillance. It is built on lines of AFP surveillance. Reporting network and machinery established for AFP surveillance is expanded to include other health facilities which are most likely to see measles, diphtheria, pertussis and NNT cases. Ministry of Health and Family Welfare, GOI has published operational field guide with technical inputs from the WHO Global VPD Surveillance Guidelines-2018.

Surveillance of tuberculosis under NTEP

- Tuberculosis has become a notifiable disease since May 2012 and notification of TB cases is made mandatory by the Ministry of Health and Family Welfare, GOI; all health facilities including government, NGOs, private clinics, hospitals and laboratories must notify all TB cases to local health authorities every month. As per "mandatory TB notification gazette for private practitioners, chemists and public health staff" issued in March 2018, all chemists who dispense TB drugs to patients will have to inform about those patients.[13]
- To facilitate TB notification, "NIKSHAY" a web enabled application which facilitates monitoring of universal access to TB patients data by all concerned" has been developed by the NTEP.[14]
- It can be used by government and private healthcare providers. All TB cases, irrespective of method of diagnosis (microbiologically confirmed or clinically diagnosed), initiation of treatment, source of treatment (government or nongovernment), type of patients (TB or DR-TB) and type of regimen used for treatment (daily or intermittent), should be notified.

Role of pediatricians / clinicians in vaccine-preventable disease surveillance

Pediatricians play a very vital role in VPD surveillance. Immediate notification of all suspected VPD cases to surveillance authorities is vital for early sample collections and laboratory confirmation or ruling out of the suspected VPD. On notification, government medical officers and / or the WHO surveillance medical officers will facilitate epidemiological case investigation and appropriate sample collection. Involvement of all healthcare providers in private sector is very important for establishment and sustaining strong surveillance system. The IAP and Indian Medical Association (IMA) must incorporate messages regarding enhancing VPD surveillance for reporting of suspected cases of VPD in all the forums.

Limitations of surveillance data

- VPD surveillance relies on symptoms and signs and may not capture asymptomatic infections. To overcome this limitation and to detect silent transmission, environmental surveillance or sero surveys are used.
- VPD surveillance depends on strong and dedicated surveillance network. Any complacency in any component of the reporting network may lead to missing the transmission of VPD under surveillance.
- Planning and monitoring vaccine introduction and effectiveness relies on strong VPD surveillance in low and middle income countries. Cost is a major barrier to VPD surveillance system maintenance and performance. There are identifiable critical gaps like cost of laboratory surveillance, challenges with costing and shared resources and missing data on capital cost.[15]

Scope of vaccine-preventable disease surveillance

- Existing surveillance system can be utilized for additional VPDs as well as other communicable disease surveillance.
- Newer advances in vaccines and laboratory diagnostic technologies can help improve existing surveillance system.

To conclude, multiple surveillance systems at national and international levels exist with specific guidelines on notification, epidemiological investigations and sample collection for laboratory confirmation. Involvement of all healthcare providers in private sector is very important for establishment and sustenance of a strong surveillance system. VPD surveillance is a dynamic process and newer advances in vaccines and laboratory diagnostic technologies improve existing surveillance system.

Points to Remember

- ***VPD surveillance is an important platform for collection of data on incidence and prevalence of vaccine preventable diseases.***
- ***This data is utilized for focused actions and interventions leading to control /eradication of infectious diseases.***
- ***It measures impact and quality of immunization programs and generates evidences for new vaccine introduction.***
- ***Multiple surveillance systems are operational in India.***
- ***All health care workers should regularly report VPDs and contribute to eradication of infectious diseases.***

References

1. World Health Organization. WHO health topics: Public health surveillance. Geneva: World Health Organization; 2014. Available from http://www.who.int/topics/public_health_surveillance/en/). Last accessed on 30 September, 2020.

2. World Health Organization. Setting priorities in communicable disease surveillance. Geneva: World Health Organization; 2006. Available from: http://www.who.int/csr/resources/publications/surveillance/WHO_CDS_EPR_LYO_2006_3.pdf?ua=1). Last accessed on 30 September 2020.
3. World health organization. Surveillance for vaccine preventable disease. Available from: https://www.who.int/immunisation/monitoring_surveillance/burden/vpd/WHO_Surveillance Vaccine Preventable_01_Overview_R2.pdf. Accessed on 30 September 2020.
4. Vashi MD, Pardeshi VR. Vaccine preventable Diseases Surveillance. In: Vashishtha VM (ed) IAP Textbook of Vaccines, 2nd edn, New Delhi, Jaypee Brothers Medical Publishers, 2020; pp64-70.
5. Mulders M, Serhan F, Goodson J, Icenogle J, Johnson B, Rota P. Expansion of Surveillance for Vaccine-preventable Diseases: Building on the Global Polio Laboratory Network and the Global Measles and Rubella Laboratory Network Platforms. J Infect Dis 2017; 216 (suppl_1):S324-S330.
6. Tate JE, Haynes A, Payne DC, Cortese MM, Lopman BA, Patel MM, et al. Trends in national rotavirus activity before and after introduction of rotavirus vaccine in to the national immunization program in the United States, 2000 to2012. Ped Infect Dis J 2013; 32(7):741-744. doi:10.1097/INF.0b013e31828d639c.
7. Von Gottberg A, de Gouveia L, Tempia S, Quan V, Meiring S, von Mollendorf C. Effects of vaccination on invasive pneumococcal disease in South Africa. N Engl J Med 2014; 371(20): 1889,1899. doi:10.1056/NEJMoa1401914NEJM. 2014.
8. World Health Organization. Frame work for verifying elimination of measles and rubella. Wkly Epidemiol Rec 2013; 88(9):89-99.
9. World Health Organization. Coronavirus disease (COVID-19) pandemic. Available from https://www.who.int/publications/i/item/who-2019-nCoV-surveillance guidance-2020.7. Last accessed on 25 November, 2020.
10. Strassburg MA. Field Epidemiology, 2nd edition. Emerg Infect Dis. 2003 Feb;9(2):280. doi:10.3201/eid0902.020697. PMCID: PMC2901956.
11. Centers for Disease Control and Prevention. Principles of epidemiology in public health practice, third edition: an introduction to applied epidemiology and biostatistics. Atlanta, USA: Centers for Disease Control and Prevention;2011. Availablefrom:https://www.cdc.gov/ophss/csels/dsepd/ss1978/index.html. Last accessed on 30 September, 2020.
12. Shastri DD, Pemde HK .VPD surveillance and IDSURV. In: Advisory Committee on Vaccines and Immunization Practices, Indian Academy of Pediatrics. IAP Guidebook on Immunization 2018-2019. Balasubramanian S, Shashtri DD, Shah AK, Pallab Chatterjee, Harish Pemde, Shivananda S, Vijaya Kumar Guduru (eds), 3rd Edn, Jaypee Brothers Medical Publishers, New Delhi, 2020; pp35-38.
13. National Guidelines on diagnosis and treatment of Pediatric Tuberculosis Available from http://www.tbcindia.nic.in/WriteReadData/l892s/3175192227 Pediatric%20guidelines_New.pdf. Last accessed on 30 September, 2020.
14. Central TB Division Ministry of Health and Family Welfare. India TB report 2020. Available from https://tbcindia.gov.in/showfile.php?lid=3544. Last accessed on 30 September, 2020.
15. Erondu NA, Ferland L, Haile BH, Abimbola T. A systematic review of vaccine preventable disease surveillance cost studies. Vaccine 2019 Apr 17; 37(17): 2311-2321. doi: 10.1016/j.vaccine.2019.02.026. Epub 2019 Mar 19. PMID: 30902482; PMCID: PMC6501781.

CLIPPINGS

Do Preterm Infants Present More Vaccine Adverse Events Than Full-Term Infants?

Studies actively monitoring adverse events following vaccination in preterm infants are scarce. This cross sectional study aimed to analyze the occurrence of adverse events associated with BCG, pentavalent, rotavirus, meningococcal C and 10-Valent pneumococcal vaccines in preterm vs. full-term infants. The study was conducted between December 2019 and March 2020. Sociodemographic and clinical variables regarding the adverse events of vaccines were assessed.

One hundred and forty-eight infants were included (74 preterm and 74 full-term). The pentavalent vaccine was more reactogenic in preterm infants since a higher frequency of adverse events (i.e., fever >38ºC and continuous or persistent crying for more than three hours) were observed (p=0.025 and p=0.004, respectively). Fever (p=0.021), irritability (p=0.043), and bloodless diarrhea (p =0.026) were also more common in preterm infants post 10-Valent pneumococcal vaccine administration. Vaccines were more reactogenic in preterm infants while on the whole, adverse effects were mild.

Rodrigues Costa ME, Abrantes Santos MEB, Costa Guedes VF, Dubourcq BC, da Fonseca Lima EJ.Do Preterm Infants Present More Vaccine Adverse Events Than Full-Term Infants? A Cross-Sectional Study. Curr Trends Vaccine Vaccinol 2020 Nov;3(1):110.

VACCINOLOGY I

UPDATE ON IMMUNIZATION FOR CHILDREN AGED 0-18 YEARS AS PER ACVIP/IAP 2020-21 RECOMMENDATIONS

** Somasundaram A*

Why this update is released?

1.New developments in vaccinology

2.Availability of newer vaccines

There are 4 major changes and 5 new additions…

Change 1: Booster dose of injectable polio vaccine at 4-6 years

A booster dose of IPV at 4-6 years of age for children who have received the initial IPV doses as per the ACVIP/ IAP schedule. In case of non-availability of standalone IPV, this dose can be administered as a combination with DPT vaccines.

Change 2: IPV mandatory in primary schedule

No child should be left without giving IPV. The most preferred option is OPV at birth, 3 doses of IPV at 6,10,14 weeks and IPV booster at 18 months and 4 - 6 years. Bivalent OPV and pentavalent only schedule should not be used in infancy without IPV (two doses of fractional dose intradermal IPV at 6 weeks and 14 weeks or a single dose1M IPV / hexavelent combination at 14 weeks). If Hexavalent vaccines are unaffordable/unavailable, the infant must be referred to a government healthcare facility for the primary immunization as per UIP schedule.

Change 3: Influenza vaccine

Uniform dosing schedule of 0.5 ml above 6 months of age for the brands which have got DCGI approval; uniform dosage recommendation shall be extended to other brands once DCGI approved.

Change 4: Varicella vaccine

The second dose of varicella vaccine should be preferably administered 3-6 months after the first dose. An earlier second dose will be beneficial over a delayed second dose.

Addition 1:Conjugate meningococcal vaccine - MENVEO

It is a quadrivalent vaccine containing the serotypes A C W135 Y and conjugated with CRM197 and recommended in children above 2years of age, adolescents and adults.

Addition 2: Conjugate Typhoid vaccine by Biological E-TYPHIBEV

Another conjugate vaccine TYPHIBEV which contains 25µg of Typhoid Vi Polysaccharide (produced from C freundii) conjugated with CRM 197 and it is approved for use between 6 months - 45 years.

Addition 3: Another monoclonal antibody (other than recommended by ACVIP 2018 - 2019) is TWINRAB for PEP of rabies

It should be used in category 3 bites along with the rabies vaccine.

Addition 4: DTaP /IPV combination vaccine -TETRAXIM

It is a fully liquid vaccine recommended for booster between 4-6 years.

Addition 5: Indigenous 10 valent Pneumococcal conjugate vaccine - PNEUMOSIL

A 10 valent vaccine by Serum Institute of India named Pneumosil has been approved with the same dosing schedule as Prevenar and Synflorix. The difference from the already existing 10 valent vaccine is that they have removed serotypes 4 and 18C and added 6A and 19A.

Excerpts taken from Recommendations by Srinivas G Kasi et al. Indian Academy of Pediatrics (IAP) Advisory Committee on Vaccines and Immunization Practices (ACVIP):Recommended Immunization Schedule (2020-21) and Update on Immunization for Children Aged 0 Through 18 Years. November 29. 2020. Indian Pediatrics.

* Consultant in Child Development and Behaviour, Sabari Child Care Center and D'Soul CDC, Chennai.

DRUG PROFILE

ORAL IRON PREPARATIONS FOR NEONATES AND CHILDREN

*** Jeeson C Unni**
**** Ranjit Baby Joseph**

Abstract: *Iron deficiency anemia (IDA) is one of the most common public health concerns globally, more so in developing countries like India. Iron deficiency in pregnancy, infancy and early childhood results in health and neurodevelopmental problems in the first 1000 days of life. Oral iron supplementation is preferred for prevention and treatment of iron deficiency anemia. This article is a review of the various oral iron preparations available in the market.*

Keywords: *Iron deficiency, Anemia, Children, Ferrous, Ferric.*

The prevalence of anemia remains very high in India despite anemia control programs, resulting in a huge burden on health, education, economy, and productivity of the nation. 'Nutritional anemia' could result from a deficiency of iron, folic acid, and vitamin B12, singly or in combination. Among these, iron deficiency anemia (IDA) is the most common and is therefore of great public health importance. The cost-effectiveness of implementing national iron supplementation programs makes it an important public health initiative[1] for improving neurodevelopmental outcomes of children in the first 1000 days of life (fetal and first two years).[2]

Table I. shows the World Health Organization (WHO) defined hemoglobin cut offs for those living at sea level.[3]

Causes of iron deficiency in children (Table II)

Children between the ages 6 and 24 months are particularly at high-risk for developing iron deficiency as most weaning foods used in our country are low in bioavailable iron. Hence, the National Consultation on Control of Nutritional Anemia has recommended targeting children in this age group for iron supplementation programs.[4] Common causes of iron deficiency among children of various age group are given in Table II.

Recommended daily allowance (RDA) of iron and sources

Dietary iron is predominantly available in two main forms: heme and nonheme. Plants and iron-fortified foods contain nonheme iron, whereas meat, seafood, and poultry contain both heme and nonheme iron.[5] The RDAs for vegetarians are 1.8 times higher than for non-vegetarians due to lower bioavailability of nonheme iron.[6] The richest sources of heme iron are lean meat and seafood and that of nonheme iron are nuts, beans, vegetables and fortified grain products. Breast milk contains highly bioavailable iron but the amounts are not sufficient to meet the needs of infants older than 4 to 6 months.[7] The bioavailability of iron is approximately 14% to 18% with mixed diets containing substantial amounts of meat, seafood and ascorbic acid; and 5% to 12% from vegetarian diets. Ascorbic acid, meat, poultry and seafood enhance absorption of nonheme iron, whereas phytate (present in grains and beans) and certain polyphenols in cereals and legumes have the opposite effect. Calcium might reduce the bioavailability of both nonheme and heme iron.[8] The RDA of iron for various pediatric age groups is given in Table III.[9]

Oral iron supplements

Current Indian drug market is flooded with more than 70000 drug formulations of iron, the majority of which are proprietary multi-drug combinations. Since hematopoiesis requires adequate supplies of minerals like iron and copper; vitamins like folic acid, vitamin B12, vitamin C, pyridoxine and riboflavin and various hematopoietic growth factors,[10] there is an indiscriminate and unscientific inclusion of several of these agents in hematinics available in the market (Table IV).[11] The cost of these combinations is also a factor that leads to noncompliance.[12] The Drugs Technical Advisory Board of India has recommended that vitamin B complex and zinc should not be included in iron and folic acid containing hematinic preparations.

* Editor-in-Chief,
IAP Drug Formulary,
Senior Lead Consultant in Pediatrics,
Aster Medcity, Kochi.
email: jeeson1955@gmail.com

** Senior Specialist in Pediatrics,
Aster Medcity, Kochi, Kerala.

Table I. Hemoglobin cut offs defining anemia at different age groups[3]

Population (gm/dL)	No anemia Hb (gm/dL)	Anemia		
		Mild anemia Hb (gm/dL)	Moderate anemia Hb (gm/dL)	Severe anemia Hb (gm/dL)
Children 6 - 59 months of age	≥11	10.0 - 10.9	7.0 - 9.9	<7
Children 5 - 11 years of age	≥11.5	11.0 - 11.4	8.0 - 10.9	<8
Children 12 - 14 years of age	≥12	11.0 - 11.9	8.0 - 10.9	<8
Non-pregnant women (15 years of age and above)	≥12	11.0 - 11.9	8.0 - 10.9	<8
Pregnant women	≥11	10.0 - 10.9	7.0 - 9.9	<7
Men (15 years of age and above)	≥13	11.0 - 12.9	8.0 - 10.9	<8

Table II. Causes of iron deficiency in children

Age groups	Risk factors
Neonate to 6 months	Low maternal iron status Prematurity, lack of iron supplementation if premature
6-12 months	Exclusive breast feeding without iron supplementation Early introduction of cow's milk before 12 months
12-48 months	Excessive intake of cow's milk (>24 ounces per day) Bottle feeds Overweight/ obese Low socio economic status
School age children	Low iron diet Obesity Gastrointestinal conditions
Adolescents	Heavy menstrual bleeding Low-iron diet Obesity High endurance sports participation (increased demands due to higher iron turnover, loss due to injury, increased sweating) Low socio economic status

Table III. Recommended daily allowance of iron in different age groups[9]

Age groups	Recommended daily allowance
7-12 months	11mg
1-3 years	7mg
4-8 years	10mg
9-13 years	8mg
14-18 years	11mg (for boys) 15mg (for girls)

Iron salts

Oral route is the preferred route of administration of iron even in case of severe deficiency. The two types of iron salts available are the ferrous and ferric forms. Since iron has to be reduced to ferrous form for absorption, ferrous salts are preferred for treatment of IDA. Other formulations are iron-amino acid chelates, iron polymaltose complexes, carbonyl iron and colloidal iron. Tablet form is considered as most stable preparation of iron. The liquid formulations are child friendly. Each iron salt contains a specific amount of elemental iron (Table V), i.e. the amount of iron in the supplement available for absorption.

A greater percentage of ingested iron is absorbed during iron deficiency states, which means that absorption of iron depends not only on type of iron salt, but also on the patient's iron deficient state.

Table IV. Different types of hematinic formulations in Indian market[11]

Types of iron supplements	Added nutrient	Examples
1. Iron salts only 2. Iron salts with vitamins 3. Iron salts with vitamins, minerals and other chemicals 4. Iron salts with minerals and other chemicals 5. Iron salts with vitamin and essential amino acids 6. Iron salts with vitamins, minerals, 7. Iron content (but without the salt being specified) 8. Iron salts with miscellaneous content	Vitamins	Vitamin A, D, E, K, C, B-complex, pantothenic acid, biotin
	Minerals and other chemicals	Zinc, copper, manganese, calcium, sodium, potassium, iodine, selenium, chromium, magnesium, phosphate, molybdate, boron, chlorine, vanadium, nickel, tin, silicon
	Essential amino acids	Histidine, lysine, glycine, glutamic acid
	Miscellaneous nutrients	Fat, protein, carbohydrate, spirulina, choline, carnitine, taurine, inositol, saffron, dioctyl sodium sulfosuccinate, lycopene, hemoglobin, succinic acid, liver extract, yeast, peptone, casein, caffeine, glycerophosphoric acid and alternate medicine products like ashwagandha, ginseng, centella asiatica.

Staining of teeth can occur with liquid preparations if they are not placed carefully at the back of the tongue. The liquid preparation may be mixed with water or juices or ingested using a straw to reduce contact with teeth. Ferrous sulphate has a salty astringent taste which is not palatable for most children.[4]

Ferrous salts

All dietary iron is absorbed in the ferrous form. Hence all the ferrous salts mentioned in Table V are ideal oral iron preparations. The commonly available ferrous salts are ferrous sulphate, fumarate, gluconate, succinate, glutamate, bisglycinate, ascorbate and citrate (injectable; ferric citrate is the oral form). These salts are amongst the cheapest preparations of iron available for medicinal use. Ferrous sulfate is not stable in its liquid form and is therefore available only as tablets. Other ferrous salts are used for making liquid formulations.

Ferrous fumarate has a similar efficacy and GI tolerance to ferrous sulphate, is moderately soluble in water, soluble in dilute acid such as gastric juice, environmentally more stable and is almost tasteless. It does not precipitate proteins and does not interfere with the proteolytic or diastatic activities of the digestive system.[13, 14]

These salts have uniformly good oral bioavailability. However, the bioavailability decreases markedly in the presence of dietary inhibitors like phytates, tannic acid, etc and hence they cannot be added to other foods/milk/fortified formulas. Some oral preparations contain ascorbic acid to aid absorption of the iron, but the therapeutic advantage of such preparations is minimal and only increase the cost of the medication. There is no justification for the inclusion of ingredients other than folic acid, in iron preparations.[14]

Although ferrous salts are highly efficacious and cheap, they have several disadvantages particularly the high incidence of gastrointestinal side effects (~23 %).[15]

Common GI side effects include constipation, diarrhea, epigastric pain, fecal impaction and dose related nausea. Certain measures suggested to minimize these side effects include administration after meals and at bed time. Decreased intestinal motility during sleep may improve absorption.[16]

Ferric salts

Ferric salts are less preferred as compared to ferrous salts as the oral bioavailability is believed to be significantly lesser.[17] They often require slightly higher dosage to produce desired therapeutic effect. Other properties are essentially similar to ferrous salts. Ferric ammonium citrate and ferric pyrophosphate are the ferric salts available in syrup form.[4]

Table V. Iron salts and the quantity of elemental iron

Iron salts	Elemental iron content
Ferrous salts	
Ferrous sulphate	20%, 300mg tablet contains 60mg
Dried ferrous sulphate	33%, 200mg contains 65mg
Ferrous ascorbate	20%, 500mg contains 100mg
Ferrous fumarate	33%, 200mg contains 66mg
Ferrous gluconate	12%, 300mg contains 36mg
Ferrous succinate	35%, 100mg contains 35mg
Ferrous calcium citrate	11%, 230mg contains 25mg
Ferric salts	
Ferric ammonium citrate	18%, 200mg contains 40mg
Ferric pyrophosphate	12%, 200mg contains 24mg
Ferric di-phosphate	30%, 120mg contains 40mg
Liposomal ferric di-phosphate	30%, 27mg contains 8mg all absorbable
Iron Amino-acid chelates	
Ferrous bisglycinate	27%, 36.5mg contains 10mg
Iron (III) hydroxide Polymaltose Complex	50mg/5ml, 100mg tablet
Colloidal Iron	32%, 250mg contains 80mg
Carbonyl iron	100% (It is 100% elemental iron as it is not an iron salt), 60mg/5ml

The newer liposomal preparations of ferric pyrophosphate, available in drops, chewable tablet and sachet forms, are however palatable, better absorbed, have minimal gastric irritation and lower teeth staining potential.[18,19] Micronization and microencapsulation of iron into liposomes is the most advanced approach to combat the dual issues of absorption and tolerance. In microencapsulation the micronized iron is encapsulated inside a lipid bilayer, similar to the biological lipid bilayer. The outer phospholipid bilayer protects the inner iron core from enzymatic degradation in the mouth or stomach and also prevents iron oxidation and degradation. This bypasses the usual route of absorption and presents almost 100% of iron to the liver where the liposome is opened by lysosomal enzymes, making the iron available for utilization.

Sucrosomial iron (SI) is another novel oral iron preparation consisting of ferric pyrophosphate protected by a phospholipid bilayer membrane. The phospholipid layer is made primarily of a sunflower lecithin and sucrester matrix. This confers SI unique structural, physicochemical and pharmacokinetic characteristics, together with high iron bioavailability and excellent gastrointestinal tolerance.[20]

Iron amino-acid chelates

Iron amino-acid chelates are conjugates of the ferrous or ferric iron with amino-acids. Although numerous conjugates have been formulated, the most studied of these are ferrous bis-glycinate, ferric tris-glycinate and ferrous glycine sulphate. Better bioavailability is claimed with these salts as absorption is not interfered by dietary phytates. Side effects with iron-amino acid chelates are less frequent.[21]

Iron (III) polymaltose complex (IPC)

It is a relatively novel iron preparation, which contains non-ionic iron and polymaltose in a stable complex. Bioavailability is almost similar to ferrous sulphate.[22] Absorption of IPC is not affected by food or milk, enabling administration without consideration of the timing of feed. It is found to have no deleterious effect on copper and zinc levels. In addition, there are no reports that it stains the teeth. However, there are several reports of inadequate or slower rise in hemoglobin.[23, 24]

Carbonyl Iron

Carbonyl iron is a small particle preparation of highly purified metallic iron that is believed to contain 100% elemental iron. 'Carbonyl' describes the process of manufacture of the iron particles (from iron pentacarbonyl gas). Carbonyl iron has been used in food fortification industry.[25] The main advantage with this form of iron is its small particle size which contributes to increased bioavailability. Another advantage is that considerably higher doses of iron can be administered. It also does not change the color or taste of the foodstuff and has high environmental stability.[26]

Dosage of elemental iron

The oral dose of elemental iron to treat deficiency is 3-6 mg/kg daily. However, smaller doses have been found to be equally effective and better tolerated. Hence a dose of 3 mg/kg/ day is currently recommended.[27] Absorptive capacity of iron in duodenum is saturated by 25 mg of elemental iron; hence higher doses will not increase the absorption.[28] The dose in adolescents is 60 mg daily.

Most of the infant formulas available are iron fortified. Therefore, the iron ingested via these feeds need to be factored while prescribing iron supplements. Preterm infants who are exclusively breastfed should be supplemented with 2mg/kg/day till one year of age and 1mg/kg/day if they are fed iron fortified formula. For term babies, supplementation is started at 4-6 months after birth with a dose of 1mg/kg/day if they are exclusively breastfed and continued until mixed feeding is established. Infants with a poor diet may become anemic in the second year of life, particularly if fed mainly cow's milk without other iron containing foods.[29]

Response to oral iron therapy in IDA is excellent. Within 1-2 days a sense of well-being ensues with decrease in irritability and improvement in appetite. A positive hematological response has been defined as rise of Hb 0.1 g/dL daily. Approximately 2 months of treatment is required for Hb to come to normal level. Two more months of therapy is required to replenish the iron stores.[2]

The hematinics market in India is currently worth around Rs. 900 crores and is growing at 15% per annum. In an India specific market survey, it was found that only 24% out of 621 formulations - 365 oral solid formulations, 232 oral liquids and 24 parenteral administration - were considered rational and acceptable.[30]

Conclusion

Iron deficiency anemia remains the most common cause of anemia in children despite various national supplementation programs. A thorough knowledge of available iron preparations is therefore necessary to ensure evidence based prescription for prevention and treatment of iron deficiency.

Points to Remember

- ***Iron deficiency anemia is extremely common in children and is of great public health importance.***
- ***Prevention, early identification and treatment of iron deficiency is essential for normal neurodevelopment.***
- ***Ferrous formulations are preferred for oral iron supplementation.***
- ***Prudent selection of appropriate formulation and awareness of elemental iron provided in each, help to ensure correct dosing.***

References

1. Kotecha PV. Nutritional anemia in young children with focus on Asia and India. Indian J Community Med 2011; 36(1):8-16. doi:10.4103/0970-0218.80786.
2. DeMayer EM, Dallmen P, Gurney JM, Hallberg L, Sood SK, Srikantia SG. Prevention of iron deficiency anaemia. In: Preventing and Controlling Iron Deficiency Anaemia through Primary Health Care. Geneva, World Health Organisation 1989;pp 34-42.
3. WHO, UNICEF, UNU. Iron deficiency anaemia: assessment, prevention and control, a guide for programme managers. Geneva, World Health Organization, 2001. Available at http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/WHO_NHD_01.3/en/index.html. Accessed on 7 November, 2020.
4. Nagpal J, Choudhury P. Iron Formulations in Pediatric Practice. Indian Pediatr. 2004; 41 (8): 807-815
5. Aggett PJ. Iron. In: Erdman JW, Macdonald IA, Zeisel SH, eds. Present Knowledge in Nutrition. 10th edn. Washington, DC: Wiley-Blackwell; 2012:pp506-520.
6. Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc: A report of the Panel on Micronutrients. Washington, DC: National Academy Press; 2001.
7. Baker RD, Greer FR. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0-3 years of age). Pediatrics 2010; 126:1040-1050.
8. Hurrell R, Egli I. Iron bioavailability and dietary reference values. Am J Clin Nutr 2010; 91:1461S-1467S.
9. National Institute of Nutrition. Dietary Guidelines for Indians - A Manual. Available from nin.res.in/downloads/Dietary Guidelines for NIN website. Accessed on 12 November, 2020.
10. Kaushansky K, Kipps TJ. Hematopoietic agents. In: Brunton LL, Lazo JS, Parker KL, editors. Goodman and Gilman's The pharmacological basis of therapeutics. 11th edn. New York: McGraw-Hill; 2006; pp1433-88
11. Karelia BN, Buch JG. Analysis of hematinic formulations available in the Indian market. J PharmacolPharmacother. 2012; 3(1):35-38. doi:10.4103/0976-500X.92504.
12. Shah A. Iron deficiency anemia-Part III. Indian J Med Sci. 2004; 58(5):214-216.
13. Rimon E, Kagansky N, Kagansky M, Mechnick L, Mashiah T, Namir M, et al. Are we giving too much iron? Low-dose iron therapy is effective in octogenarians. Am J Med 2005; 118:1142-1147.
14. Joint Formulary Committee. British National Formulary for children. London: BMJ Group and Pharmaceutical press, 2013-2014; pp443-446
15. Hallberg L, Ryttinger L, Solvell L. Side Effects of oral iron therapy. A double blind study of different iron compounds in tablet form. Acta Med Scand Suppl 1966; 459: 3-10.
16. Andrews NC. Disorders of Iron Metabolism and Sideroblastic Anemia. In: Nathan DG, Ginsburg D,

Orkin SH, Look AT. Nathan and Oski's Hematology of Infancy and Childhood. Philadelphia: WB Saunders; 2003; pp 455-497.

17. Santiago P. Ferrous versus Ferric Oral Iron Formulations for the Treatment of Iron Deficiency: A Clinical Overview. Scientific World Journal 2012; 846824. doi: 10.1100/2012/846824.
18. Fidler MC, Walczyk T, Davidsson L, Zeder C, Sakaguchi N, Juneja LR, et al. A micronised, dispersible ferric pyrophosphate with high relative bioavailability in man. Br J Nutr 2004; 91(1):107-112. [PubMed:1474 8943]
19. Hussain U, Zia K, Iqbal R, Saeed M, Ashraf N. Efficacy of a Novel Food Supplement (Ferfer®) Containing Microencapsulated Iron in Liposomal Form in Female Iron Deficiency Anemia. Cureus2019, 11(5): e4603.DOI: 10.7759/cureus.4603
20. Brilli E, Romano A, Fabiano A, Zambito Y, Di Raimondo F, Tarantino G. Sucrosomial technology is able to promote ferric iron absorption: pre-clinical and clinical evidences. Blood 2016;128(22):3618.
21. Pineda O, Ashmead HD. Effectiveness of treatment of iron-deficiency anemia in infants and young children with ferrous bis-glycinate chelate. Nutrition 2001; 17: 381-384.
22. Borbolla JR, Cicero RE, Dibildox MM, Sotres DR, Gutierrez RG. Iron polymaltose complex vs. iron sulphate in the treatment of iron deficiency in infants. Rev Mex Pediatr 2000; 67: 63-67.
23. Mehta BC. Ineffectiveness of iron polymaltose in treatment of iron deficiency anemia. J Assoc Physicians India 2003; 51: 419-421.
24. Nielsen P, Gubbe EE, Fischer R, Heinrich HC. Bioavailability of iron from ferric polymaltose in humans. Drug Research1994; 44: 743-748.
25. Department of Health and Human Sciences Food and Drug Administration. Iron-containing supplements and drugs: label warning statements and unit-dose packing requirements.Final rule. Federal Register 21CFR parts 101,111 and 310., 1997; 62(10):p2218.
26. Gordeuk VR, Brittenham GM, McLaren CE, Hughes MA, Keating LJ. Carbonyl iron therapy for iron deficiency anemia. Blood 1996; 67 (3): 745-752.
27. Choudhury P. Iron deficiency anemia. In: Lokeshwar MR, Shah NK, Agarwal B, Sachdev A, editors. IAP Speciality Series on Pediatric Hematology and Oncology. Mumbai: Indian Academy of Pediatrics, 2006; pp 28-35.
28. Andrews NC. Disorders of Iron Metabolism and Sideroblastic Anemia. In: Nathan DG, Ginsburg D, Orkin SH, Look AT. Nathan and Oski's Hematology of Infancy and Childhood. Philadelphia: WB Saunders; 2003; pp 455-497.
29. Wang M. Iron Deficiency and Other Types of Anemia in Infants and Children. Am Fam Physician 2016; 93(4):270-278.
30. Karelia BN, Buch JG. Analysis of hematinic formulations available in the Indian market. J Pharmacol Pharmacother 2012; 3(1):35-38.

CLIPPINGS

Phase 2b Controlled Trial of M72/AS01$_E$ Vaccine to Prevent Tuberculosis.

A randomized, double-blind, placebo-controlled, phase 2b trial of the M72/AS01E tuberculosis vaccine was conducted in Kenya, South Africa and Zambia. Human immunodeficiency virus-negative adults 18 to 50 years of age with latent M. tuberculosis infection (by interferon-γ release assay) were randomly assigned (in a 1:1 ratio) to receive two doses of either M72/AS01E or placebo intramuscularly 1 month apart. Most participants had previously received the Bacille Calmette Guérin vaccine.

The safety of M72/AS01E and its efficacy against progression to bacteriologically confirmed active pulmonary tuberculosis disease was assessed. Primary analysis (conducted after a mean of 2.3 years of follow-up) of the ongoing trial was reported. A total of 1786 participants received M72/AS01E and 1787 received placebo and 1623 and 1660 participants in the respective groups were included in the according-to-protocol efficacy cohort. A total of 10 participants in the M72/AS01E group developed bacteriologically confirmed active pulmonary tuberculosis as compared with 22 in the placebo group (incidence, 0.3 cases vs. 0.6 cases per 100 person-years). There were more adverse events in the M72/AS01E group (67.4%) than in the placebo group (45.4%) within 30 days after injection, with the difference attributed mainly to injection-site reactions and influenza-like symptoms. There was no difference in serious adverse events, potential immune-mediated diseases and deaths.

M72/AS01E provided 54.0% protection for M. tuberculosis-infected adults against active pulmonary tuberculosis disease, without evident safety concerns.

Van Der Meeren O, Hatherill M, Nduba V, Wilkinson RJ, Muyoyeta M, Brakel EV, et al.Phase 2b Controlled Trial of M72/AS01E Vaccine to Prevent Tuberculosis. N Engl J Med 2018; 379:1621-1634.

DERMATOLOGY

TOPICAL CORTICOSTEROIDS IN CHILDREN - AN OVERVIEW

** Vijayabhaskar C*
** Madhu R*

Abstract: *Topical corticosteroids are extensively used in steroid responsive pediatric dermatoses by virtue of anti-inflammatory, antiproliferative and immunosuppressive effects. They are classified into various groups depending on the potency of the molecule. In pediatric age group least potent to mid potent topical corticosteroids are used depending on the age and site. The quantity, duration of application, vehicle and concentration of the molecule determine the outcome of the disease and prevention or reduction of the adverse effects. Vehicles in different forms are used for different anatomical sites. In chronic conditions, when steroids are to be used for longer period of time one has to judiciously taper the potency of the corticosteroid molecule, reduce the frequency of application or change to non-steroidal formulation in order to reduce the adverse effects. There is a need to address the issue of corticosteroid phobia which is quite often observed among physicians and parents.*

Keywords: *Corticosteroids, Potency, Vehicle, Steroid phobia.*

Corticosteroids, synthetic drugs used for many diseases mimic cortisol, which is naturally produced by adrenal glands. Topical corticosteroids are a group of topical agents with anti-inflammatory effect which are used extensively in pediatric dermatoses. In 1952, Sulzberger and Witten used topical steroids for the first time in eczematous dermatitis successfully.[1] Through the years, different types of molecules of topical steroids have become available in different concentrations and potencies to treat various cutaneous disorders. However, one has to ensure the judicious use of these molecules in the treatment of steroid responsive dermatoses. But in common practice, there is often a tendency to misuse or abuse, due to lack of knowledge about these molecules which could result in significant adverse effects. At the same time, in contrast, steroid phobia also exists among parents who refuse to use corticosteroids even when indicated resulting in treatment failure.[2]

* Associate Professor,
Department of Dermatology,
Madras Medical College,
Chennai.
email: buskibuski@gmail.com

Mechanism of action

Corticosteroids inhibit the endogenous inflammatory mediators such as kinin, histamine, liposomal enzymes and prostaglandins resulting in vasoconstriction, immunosuppression, anti-proliferative and anti-inflammatory action. In addition, these molecules exert antimitotic activity on tissues including the epidermis.[3]

Classification of topical corticosteroids

The topical steroids are classified according to the potency into 7 classes (Table I).[4] In pediatric population, super potent and potent topical steroids like halobetasol propionate, clobetasol propionate and mometasone ointment are not to be used, whereas mometasone, fluticasone, desonide and hydrocortisone creams could be used.[5] The effects and adverse effects of topical corticosteroids (TCS) depend on the age, potency, quantity, frequency, duration, vehicle and the site of application.[4,6]

Factors that impact effects of TCS

Age

The US FDA has approved the use of desonide and 1% hydrocortisone creams in children above 3 months of age.[7,8] Safety of fluticasone molecule in infants above 3 months of age has been documented.[9] Mometasone cream are to be used in children aged above two years.[10,11] Superpotent steroids like clobetasol propionate and halobetosol propionate are not to be used in children below 12 years of age.[5,12] FDA approves the use of clobetasol molecule, above 12 years of age, while use of halobetasol is not approved. As per classification, topical steroids from Class III to Class VII can be used safely in children.

Potency

The potency of the topical steroid is assessed by its vasoconstrictive properties.[6] Based on this effect, the topical steroids are classified into various categories.

Table I. Classification of topical corticosteroids based on the potency

Class		Topical corticosteroid
I	Super potent	Clobetasol propionate 0.05% cream/ointment Halobetasol propionate 0.05% cream/ointment Fluocinonide 0.1% ointment Betamethasone dipropionate 0.05% ointment
II	Potent	Mometasone furoate 0.1% ointment Betamethasone dipropionate 0.05% cream Fluocinonide 0.05% ointment Halcinonide 0.1% cream
III	Upper mid strength	Betamethasone dipropionate 0.05% lotion Triamcinolone acetonide 0.1% ointment Fluticasone propionate 0.005% ointment Halometasone 0.05% cream
IV	Mid strength	Mometasone furoate 0.1% cream/lotion Flucinolone acetonide 0.25% ointment
V	Lower mid strength	Betamethasone valerate 0.1% cream Fluticasone propionate 0.05% cream Hydrocortisone butyrate 0.1% cream Flucinolone acetonide 0.25% cream
VI	Mild	Desonide 0.05% cream Fluocinolone acetonide 0.01% cream Triamcinolone acetonide 0.025% cream
VII	Least potent	Hydrocortisone acetate 1%, 2.5% cream/lotion

The American system of classification is given in Table I. It ranges from Class I to Class VII, where Class I is super potent and class VII is the least potent steroid.

Absorption of TCS in different sites

The anatomical site, characteristic features of the stratum corneum and lipid content of the skin determine the penetration and absorption of the topical steroid. Penetration and absorption is least over the palms, soles and forearms which is around 0.8 to 1%, while it is 4% in the intertriginous area, 10% over the face and 40% over the scrotum and eyelids. In children, mid potent steroids may be used over palms and soles while only mild steroids are to be used over the face. Similarly, only least potent steroids are to be used in the intertriginous areas, as application of medicines in these sites tend to result in occlusive effects.[13] Mild and least potent steroids are safe to be used over larger surface areas of the body.[6]

Quantity of application

The quantity of topical steroids to be used is determined by fingertip unit. One fingertip unit is the amount of the drug drawn from the distal crease to the tip of the index finger of an adult from a standard tube of medicine whose nozzle is 5mm in diameter.[14] This is roughly equivalent to 0.5grams which can be applied on an area equivalent to twice the size of the adult palm with all fingers in close approximation.[15] Study by Nelson AA, et al done to find the quantity of corticosteroid required in the various age groups, concluded that one-fifths of the adult dose is required in infants, two-fifths in children and two-thirds in adolescents.[16]

Frequency of application

Most of the topical steroids used in children are to be applied once or twice daily, depending on the condition to get the desired result.[4] Mometasone cream has to be used only once daily.[10]

Duration

Topical corticosteroids can be used safely for 2 weeks in children and have to be reviewed. However, the mild and least potent steroids namely desonide and hydrocortisone creams respectively can be used safely up to 4 weeks.[7,8] It should be insisted that the duration should not exceed that recommended by the physician in order to prevent adverse effects.

Vehicle, concentration and occlusion

Topical corticosteroids are available in cream, ointment, lotion and gel formulations. Selection of the vehicle depends on the nature of the skin condition. In steroid responsive oozy lesions, cream is preferred as it has a drying effect and is cosmetically acceptable. In dry, chronic conditions, ointments are preferred over creams as they have lubricating and occlusive effect. Folliculitis can occur if ointments are used in hairy regions. In hairy regions, either gel or lotions are preferred as they are less greasy and have good occlusive effect. Moreover, they penetrate well and do not leave any residue. Ointments are preferred over palms and soles, as the skin is thick and penetration of the steroid is better with ointment.[15,17]

Any steroid applied under occlusion will enhance the penetration of the molecule. When steroids are applied under occlusion, drug stays in the stratum corneum for 2 weeks resulting in a reservoir effect, whereas when applied without occlusion, drug effect lasts for 2 days.[18] Studies have documented that wet wrap dressings with diluted corticosteroids have been found to be effective in children with severe and or refractory atopic dermatitis.[19] Axillae and groin in children and adolescents, and infra mammary region in adolescent age group tend to have a self- occlusive effect and hence only mild potent topical steroids are to be used and ointments are best avoided.[4]

A steroid molecule will have different potency depending on the vehicle used. Topical steroid in a cream formulation is less potent than the same molecule in ointment form. Concentration of steroids also play a major role in deciding the efficacy. The more concentrated a molecule is, the more potent effect it has. Same concentration of the steroid in different vehicles, has different potency.[13, 17]

Site

The potency of the steroid also depends on the site of application. Over the face and flexures like neck, axillae and groin, mild potent steroids are preferred as these sites are occlusive and tend to increase the potency of the steroid. Hence, mild potent steroids like desonide and hydrocortisone are preferred for application on the face and flexures. Potent steroids like mometasone and fluticasone are preferred for use over the palms and soles.[15]

Tapering or withdrawal of topical steroids

In chronic conditions like atopic dermatitis, psoriasis, lichen planus, chronic eczema etc, after daily application for a period of 2 to 4 weeks, steroids should be either used intermittently or be tapered with less potent steroids for maintenance.[20] In intermittent therapy, the same molecule is used on alternate days for the next 2 weeks and later it can be tapered to twice a week for the next 2 to 4 weeks and may be stopped. In conditions like atopic dermatitis, once the desired results are achieved, potency of the steroids can be tapered to weekend application for 2 days in a week with emollients being applied on the other days.[21] Steroid sparing agents like calcineurin inhibitors (Tacrolimus or pimecrolimus) may be substituted for maintenance purpose.[22] When there is an exacerbation of the lesion, topical steroids may be reintroduced.

Adverse effects

Use of potent steroids or prolonged usage of topical steroids can have local and systemic side effects. Prescribing physician has to be aware of the side effects of topical steroids and it is better to educate the parents and or the patients in case of adolescents about the need for adherence to treatment as per the advice and adverse effects. When the parents or the patients notice the early adverse effects, they can stop application and seek medical advice.

The various local side effects that can occur are secondary infection (bacterial, fungal or viral) exacerbation of existing fungal infection, aggravation of viral infections, atrophy, telangiectasia, purpura, hypopigmentation, hyperpigmentation, hypertrichosis, acneiform eruptions, perioral dermatitis, striae, steroid addiction and allergic contact dermatitis.[23] Steroids when applied on the bacterial lesions can aggravate the bacterial growth and lead to exacerbation. In superficial fungal infections, steroids when applied topically give symptomatic itch relief but fungus continues to grow with exacerbation of lesion leading to steroid modified tinea and tinea incognito. When used near the eyes, mid potent steroids even for shorter duration or low potent molecule for long duration, may lead to glaucoma and cataract.[2] Atrophy of the skin could result either due to improper usage of high potent topical steroids even for a short period of time or prolonged usage of any steroids. When steroid responsive dermatoses are not responding to topical steroids, it could indicate an allergic contact dermatitis to steroid molecule itself.[24]

Children are more susceptible to systemic adverse effects of topical corticosteroids due to the increased ratio of body surface area to body weight compared to adults. Prolonged topical application of steroids could result in suppression of the pituitary adrenal axis, growth retardation, hypertension, hyperglycemia, Cushing's syndrome, etc.[4,24,25]

Combination of steroids

Topical corticosteroids in combination with antibacterials, antifungals, antiseptics, etc are widely available over the counters. These combinations are irrational and should not be used. The few dermatoses, wherein combination of topical steroids with antibacterials could be used are subacute eczema and atopic dermatitis. Triple, four and five drug combination creams of topical corticosteroids with antifungals and antibacterials are strictly to be avoided as these creams will worsen the skin condition and cause deleterious adverse effects on the skin.[26]

Decision on clinical suspicion

When one encounters skin lesions, wherein a differential diagnosis of steroid responsive dermatosis and fungal infection are considered, it is better to refer the patient to a dermatologist or if none available, to err on the fungal side. Initiation of treatment with topical antifungals for 2 weeks could be followed by review. If there is response, the same regimen may be continued. In case of non-response, steroids could be started.

Topical corticosteroid phobia

Phobia is an irrational fear of something that leads to aversion and deliberate avoidance of it. Steroid phobia is very common among the treating physicians and patients or the care givers in pediatric practice. Many a times, magnitude of adverse effects caused by systemic steroids is confused with that produced by topical corticosteroids. The care giver or the adolescent patient should be explained about the steroid responsive dermatoses and the necessity to use steroids in such conditions. They should be clearly taught that mere application of steroids will not cause side effects. They should be counselled to strictly follow the physician's advice regarding the amount and duration of application of steroids.[27]

Referring to the dermatologist

Most of the pediatric patients and parents are more comfortable with their pediatricians than a referral dermatologist. Steroid responsive dermatoses like contact dermatitis, mild form of atopic dermatitis, pityriasis alba, polymorphous light eruption, lichen striatus, allergic/irritant contact dermatitis, etc may be treated by pediatricians, with due attention to the potency of steroid and duration to be used. In case of uncertainty in clinical diagnosis, therapeutic non-response at the end of 2 weeks after starting treatment or exacerbation of previously responding dermatosis, it is better to refer to the dermatologist. Children with chronic skin conditions like psoriasis, vitiligo, refractory alopecia areata and moderate to severe atopic dermatitis, etc may be referred to the dermatologists.

Conclusion

Topical corticosteroids, if used judiciously will result in successful management of steroid responsive dermatoses. Various factors like the site, amount, potency, vehicle, frequency and duration of topical steroid determine the efficacy of the molecule. Education of the patients, care givers and practicing physicians will help in reduction of the side effects of topical corticosteroids and elimination of steroid phobia.

Points to Remember

- ***Mild and least potent topical corticosteroids are to be used for infants and mid to moderate potent steroids in children.***
- ***Least potent steroids are safe for use in the flexural areas***
- ***Desonide or hydrocortisone cream can be used over the face.***
- ***Mometasone cream is to be used above two years of age.***
- ***Creams to be used over the body and face and ointment over the thick regions like palms and soles***
- ***Duration of application is usually for 2 weeks - 4 weeks in case of least potent steroids. Then taper the potency or change to intermittent application as per clinical scenario.***
- ***Parents and adolescent patients have to be counselled about compliance to treatment, adverse effects and steroid phobia.***

References

1. Sulzberger MB, Witten VH. The effect of topically applied compound F in selected dermatoses. J Invest Dermatol. 1952; 19:101-102.
2. Coondoo A, Phiske M, Verma S, Lahiri K. Side-effects of topical steroids: A long overdue revisit. Indian Dermatol Online J 2014; 5:416-425.

3. Gabros S, Nessel TA, Zito PM. Topical Corticosteroids. [Updated 2020 Sep 29]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK532940/ Last accessed on 10 November, 2020.
4. Rathi SK, D'Souza P. Rational and ethical use of topical corticosteroids based on safety and efficacy. Indian J Dermatol 2012; 57:251-259.
5. Coondoo A, Chattopadhyay C. Use and abuse of topical corticosteroids in children. Indian J Pediatr Dermatol 2014; 15:1-4.
6. Ference JD, Last AR. Choosing Topical Corticosteroids. Fam Physician 2009; 79:135-140.
7. US Food and Drug Administration. Center for Drug Evaluation and Research. Desonate gel prescribing information. Available from: URL:https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/021844s008lbl.pdf. Last accessed on 28 November, 2020.
8. US Food and Drug Administration. Center for Drug Evaluation and Research. FDA drug application20 2145 by Glenmark generics. Available from: URL: https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/202145Orig1lbl.pdf. Last accessed on 28 November, 2020.
9. Fried Lander SF, Hebert AA, Allen DB. Safety of fluticasone propionate cream 0.05% for the treatment of severe and extensive atopic dermatitis in children as young as 3 months. J Am Acad Dermatol 2002; 46(3):387-393.
10. US Food and Drug Administration. Center for Drug Evaluation and Research. Soltamox prescribing information. Available from:https://www.accessdata.fda.gov/drugsatfda_docs/label/2002/19625s12s13lbl.pdf. Last accessed on 2 December, 2020.
11. US Food and Drug Administration. Center for Drug Evaluation and Research. Mometasane furoate prescribing information. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/019543s024lbl.pdf. Last accessed on 2 December, 2020.
12. US Food and Drug Administration. Center for Drug Evaluation and Research. Available from:https://www.accessdata.fda.gov/drugsatfda_docs/anda/2001/75733_Clobetasol%20Propionate_Prntlbl.pdf. Last accessed on 5 December, 2020.
13. Carlos G, Uribe P, Penas PF. Rational use of topical corticosteroids. Aust Prescr 2013; 36:158-161
14. Long CC, Finlay AY. The finger-tip unit: A new practical measure. Clin Exp Dermatol 1991; 16:444-447
15. Topical therapy and topical corticosteroids. In: Habif TP, editor. Clinical Dermatology. 5thedn. New Delhi: Mosby Elsevier Inc; 2010; pp75-90.
16. Nelson AA, Miller AD, Fleischer AB, Balkrishnan R, Feldman SR. How much of a topical agent should be prescribed for children of different sizes? J Dermatolog Treat 2006; 17(4): 224-228.
17. Maronn ML, Bree AF, Siegfried EC, Zvulunov A, Zinman OW, Amitai DB, et al. Principles of treatment in pediatric dermatology. In: Schachner LA, Hansen RC, editors. Pediatric Dermatology. 4thedn, Vol I. China: Mosby Elsevier; 2011; pp 146-148.
18. Saraswat A. Ethical Use of Topical Corticosteroids. In: Lahiri K. (eds) A Treatise on Topical Corticosteroids in Dermatology. Springer, Singapore.2018;73-79. https://doi.org/10.1007/978-981-10-4609-4_5. Last accessed on 5th December, 2020.
19. Devillers ACA, Oranje AP. Efficacy and safety of 'wetwrap' dressings as an intervention treatment in children with severe and/or refractory atopic dermatitis: a critical review of the literature. 2006; 154(4):579-585.
20. Mehta AB, Nadkarmi NJ, Patil SP, Godse KV, Gautam M, Agarwal S.Topical corticosteroids in dermatology. Indian J Dermatol Venerol Leprol 2016; 82:371-378.
21. Jeziorkowska R, Jedrzejowska AS, Samochocki. Topical steroid therapy in atopic dermatitis in theory and practice. Postepy Dermatol Alergol 2015; 32(3):162-166.
22. Saeki H, Furue M, Furukawa F, Hide M, Ohtsuki M, Katayama I, et al. Guidelines for management of atopic dermatitis. J Dermatol 2009; 36:563-577.
23. Abraham A, Roga G. Topical steroids damage skin. Indian J Dermatol 2014; 59(5):456-459.
24. Saraswat A. Topical corticosteroids use in children: Adverse effects and how to minimise them. Indian J Dermatol Venereol Leprol 2010; 76:225-228.
25. Tiwari A, Goel M, Pal P, Gohiya P. Topical steroid induced iatrogenic cushing syndrome in the pediatric age group: Rare case report. Indian J Endocr Metab 2013;17: S257-S258.
26. Verma SB. Topical corticosteroids misuse in India is harmful and out of control. BMJ 2015;351:h6079 doi:10.1136/bmj.h6079.
27. Coondoo A, Sengupta S. Topical corticophobia among parents and caregivers of atopic children. Indian J Pediatr Dermatol 2016; 17:255-257.

ERRATUM

Article titled "Antifungals in children" Indian J Pract Pediatr 2019; 21(1):65-76.

Page No.69: Tinea pedis / Tinea manuum: Child 1 month - 11 years: To rewrite as - "Itraconazole 3 - 5 mg/kg" in place of 35 mg/kg once daily (max. per dose 100 mg) orally for 30 days

Editorial Board
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GENERAL ARTICLE

APPLICATION OF FLOW CYTOMETRY IN PEDIATRIC HEMATOLOGY / ONCOLOGY

** Aruna Rajendran*
*** Thilagavathi V*

Abstract: *Flow cytometry (FC) is a laser-based technology which is used to detect and measure physical and chemical characteristics of a population of cells or particles. It is a tool for rapid analysis, where thousands of cells can be quickly examined and processed by a computer. It is highly useful in the study of immune dysfunction and hematological malignancies. In the last 60 years, millions of HIV infected patients in resource poor environments are living longer through therapy management guided by flow cytometry. It is also useful in the diagnosis of many rare but benign illness like paroxysmal nocturnal hemoglobinuria. Great benefit of flow cytometry is the ability to test large number of cells in a short time. It has lot of applications in diagnostics and recently flow cytometry assays have been developed to identify parasites such as cryptosporidium and giardia. This article covers the principles of flow cytometry - Optics, Fluidics ad Dynamics, its diagnostic applications and limitations in present use.*

Keywords: *Hematological malignancy, Minimal residual disease, Immune deficiency.*

Flow cytometry has evolved as a major technique in establishing the diagnosis and prognosis of hematological diseases. It also helps in deciding targeted use of monoclonal antibodies based on cluster of differentiation (CD) expression by malignant cells. Availability of flowcytometry in many institutions has made accurate and early diagnosis of acute leukemias possible, enabling early treatment initiation and better outcomes. With identification of CD markers, flowcytometry is now helpful in the diagnosis of many benign hematological and immunological disorders and in stem cell transplant. In this review, we will go through principles, diagnostic application, limitations of flowcytometry and case scenarios.

* Assistant Professor,
Department of Pediatric Hematology Oncology,
Institute of Child Health and Hospital for Children,
Chennai & Bone Marrow Transplant Physician

** Senior Consultant, Hemato-Oncologist,
Vasantha Subramanian Hospital, Chennai.

email: drarunasasidaran@gmail.com

I. Principles Flow cytometry analyses various qualitative and quantitative parameters of a cell such as cell size and cellular contents, for cell sorting. Cells present in liquid media like bone marrow, peripheral blood, cerebrospinal fluid, pleural fluid, peritoneal fluid, ascitic fluid and cell suspension from a lymph node aspirate can be used for flowcytometric evaluation. It can analyse several thousands of cells per second.[1] It can study around twenty features of a cell simultaneously. Flow cytometry has 4 major components (Fig.1).

1. Fluidics - generates a sheath fluid consisting of single cells
2. Excitation optics - consists of multiple lasers
3. Collection optics - transmits the emitted wavelengths to the photomultiplier tube (PMT)/ charge coupled devices (CCD)/ detector arrays in multiple angles
4. Analyzer - transforms electrical signals to digital signals and displays quantitative and qualitative data

Fluorescent dyes or fluorophore conjugated antibody are used to label various cellular contents. Cells in suspension (fluidics) travel in a single stream, passing individually through a point where a light beam is focussed (excitation optics). Cells scatter light and fluorochrome conjugated antibody is excited by the light beam. Fluorescein isothiocyanate (FITC), Texas red and phycoerythrin (PE) are the most commonly used fluorescent dyes. Each fluorochrome has a specific excitation spectrum and gets excited at a specific wavelength. Once excited, it will release a photon of light which will be unique to different types of fluorochromes. Collection optics collects each particular fluorochrome light from all ranges of emitted spectra. It consists of multiple detectors focussed at the intersection of light beam and cell stream. One detector is placed in the line of light beam (forward scatter) and several detectors perpendicular to it (side scatter) along with multiple fluorescence detectors (Fig.1).

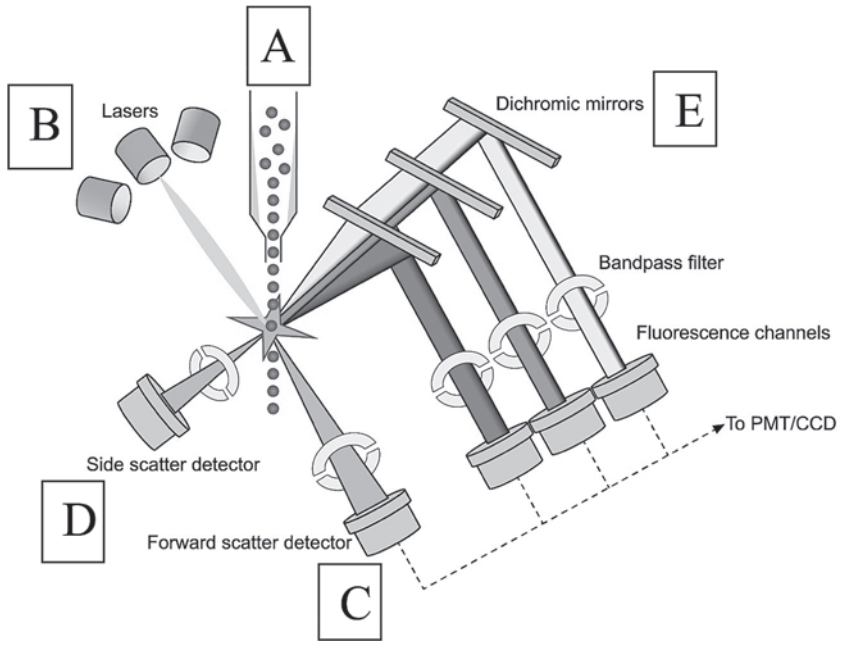


Fig.1. Mechanism of Flow cytometry[1]

Single sheath of cells (A) suspended in fluid is being intersected by a beam of Laser Light (B) Intersection of single cell and laser light leads to scattering of light. This scattered light is detected by forward (C) and side scatter detector (D) Forward scatter detector sorts cells by cell size and side scatter detector sorts cells by internal complexity (granularity). Fluorescent dyes conjugated with antibodies are activated by different types of laser and emit a specific wavelength which are detected by detectors with dichromic mirrors (E) Current flow cytometers have 4 lasers and ≥ 18 detectors which can study up to 4 antigens in one cell simultaneously. The signals detected are amplified and displayed on screen for analysis (modified figure from Woo J et al, Expert Rev Mol Diagn 2014).

Forward scatter detector measures the size of a cell and side scatter assesses the granularity/content of the cell.[2] With these physical properties, it becomes possible to assort the cells into lymphocytes, granulocytes and reticulocytes. The emitted light is separated into a specific wavelength light when the optics collect the light and direct it to a series of filters and dichromic mirrors. These detectors digitize signals for computer analysis.[1] The information is displayed as a Histogram or 2D/ 3D plots. Based on fluoroscent intensity and CD expression, gating (eg. CD 45 gating in acute leukemias) can be done and specific cell population can be studied.

Clusters of differentiation (CD) expression (Table I)

Clusters of differentiation (CD) represent the cell surface markers of hematopoietic differentiation indicating lineage of the cell. There are >400 CD antigens identified. Non-committed hematopoietic cells express CD 34, CD 38 and HLA-DR. TdT (Terminal deoxynucleotidyl Transferase) is expressed by lymphoid precursors. Normal B cells express surface immunoglobulins along with CD19, C20 and CD22. T cells are identified by the lineage specific marker Cytoplasmin CD3. Similarly the most specific myeloid marker is myeloperoxidase (Table I). Monocytic differentiation is identified with expression of CD11 and CD14. Flowcytometry helps in identifying cell lineage based on CD expression.[3]

Fluoroscent dyes can get incorporated into DNA, enabling detection by flow cytometry in millions of cells at one time point. Based on the DNA and RNA content, it can be used to understand the DNA ploidy (ploidy is the number of complete sets of chromosomes in a cell) and cell cycle. Dyes used for these studies include - propidium iodide (PI, most widely used), 42, 6-diamidino-2-phenyl indole (DAPI, DNA specific), DRAQ5 (one of the Vybrant Dye Cycle compounds or Hoechst 33342 for viable cells) and thiazole orange (RNA specific). This information is used in cancer diagnosis (DNA ploidy) and to study reticulocytes and platelets (with RNA specific agents).[4] Hyperdiploidy is associated with a good outcome and hypodiploidy is associated with a poor outcome in acute lymphoblastic leukemia.

Table I. Common antigens for diagnosis of hematological malignancies and common blood cell types[1,3]

Disease condition	**Common CD (cluster differentiation) markers**
B cell ALL	CD 19, CD 20, CD 22, CD 79 a, CD 10, PAX 5, CD 45, TdT
T cell ALL	CD 3, Cytoplasmic CD 3, CD 4, CD 8, CD 5, CD 7, CD 2, CD 45
AML	CD 13, CD 33, CD117, MPO, HLA DR, CD 34
Paroxysmal nocturnal hemoglobinuria (PNH)	CD 55, CD59
Leukocyte adhesion defect	CD11, CD18
Type of cell	**Identification marker**
Hematopoietic stem cell	CD 34, CD 43, CD 17, CD 45, CD 133
NK cells	CD 16, CD 56
Granulocytes	CD 13, CD 14, CD 15, CD 33, CD 45, CD 10, MPO
Monocytes	CD 13, CD 14, CD 33, CD 64, CD 68, CD 45
Megakaryocytes/ Platelets	CD 41, CD 61, CD 31, CD 36, CD 71, CD 45
Erythrocytes	CD 36, CD 41, CD 71, CD 235, Glycophorin A
Myeloblasts	CD 13, CD 33, CD 117, CD 38, HLA DR, CD 34, CD 45
Promyelocytes	CD 13, CD 15, CD 33, CD 117, MPO, CD 45
Gamma - Delta T cell	TCR$\gamma\delta$, CD 3
Helper T cell	TCR$\alpha\beta$, CD3, CD4
Cytotoxic T cell	TCR$\alpha\beta$, CD3, CD8
Mature B Cell	CD 19, CD 20, IgM, IgD
Memory B cell	CD 19, CD 20, IgG, IgA, or IgE
Double negative T Cell	Pre-TCR, CD3
Double positive T Cell	TCR$\alpha\beta$, CD 3, CD4, CD8

II. Diagnostic applications (Table II)

A. Leukemia and lymphoma

i. **Diagnosis:** Acute lymphoblastic leukemia (ALL) constitutes 80% and acute myeloid leukemia (AML) constitutes 20% of leukemia in children. Leukemic cells exhibit aberrant CD expression on the cell surface or intracellularly as compared to normal hematogones. Flowcytometer analyses these antigens in a short period of time (4 to 6 hours time). The equivalent test of flowcytometric analysis is the study of surface antigens by immunohistochemistry in a bone marrow biopsy sample which will take at least 5 days for interpretation. Establishing early diagnosis in leukemia and lymphomas, which are high grade tumours is crucial for initiating early treatment.

Flow cytometry helps in subclassifying leukemia broadly into ALL and AML. ALL are further classified into subtypes of B cell ALL [Precursor B-ALL-most common; Mature B - ALL (Burkitt's)] and T cell ALL (T-ALL and Early Precursor T-ALL). AML is sub classified into eight categories from M0 to M7. Flowcytometry has helped in typing of difficult AML subtypes which are cytochemical stain (Myeloperoxidase) negative such as minimally differentiated subtypes (AML M0 & M1) and acute megakaryoblastic leukemia (AML M7).[5] Similarly, diagnosis of mixed lineage leukemia (MLL) / biphenotypic leukemia which co-expresses multiple lineage (e.g. B ALL with myeloid co-expression) which often carries poor prognosis, has been made possible with the availability of flowcytometry.

Table II. Applications of flow cytometry in Pediatric Hematology Oncology[1]

Hematological malignancies
Diagnosis and subclassfication of acute leukemia and non-Hodgkins lymphoma
DNA ploidy studies - Risk stratification of acute leukemia
Proliferation Index (Ki-67)
CD 20 testing to assess Rituximab usage potential in lymphoma/ leukemia
Minimal Residual Disease to assess molecular remission in LeukemiasPediatric solid tumours like neuroblastoma and Ewings sarcoma.[5]
Immuno deficiencies
Serial CD4 counts in HIV disease monitoring
Lymphocyte subset analysis in diagnosis of severe combined immune deficiency
CD 11 and CD 18 enumeration in LAD diagnosis
DHR assay for chronic granulomatous disease diagnosis
Double negative T cell assessment in autoimmune lymphoproliferative disorder diagnosis
Cytokine protein identification - IL-2, TNF-α, IFN-γ
Benign hematology
Diagnosis of PNH using CD 55 and 59 assessment
Flowcytometric EMA study for RBC membrane disorders like hereditary spherocytosis
Identification of fetal RBC in maternal blood – diagnosis and quantification in feto maternal hemorrhage
Hematopoietic stem cell transplant
Stem cell content (CD34) enumeration in hematopoietic stem cell product for cell dosing in bone marrow transplant and in umbilical cord blood stem cell cryopreservation
Cell viability assessment in stem cell products after thawing from storage
TCR $\alpha\beta$ and B cell depletion for haplo-identical bone marrow transplantation
Immunology
HLA B 27 identification in spondyloarthropathies
Blood banking
Red cell typing of recipient cells for blood grouping
Quantification of leukocyte content in the blood product

ii. **Prognosis and therapeutic targets:** Flow cytometry also helps in identifying prognostic markers and markers for therapeutic use. For example, with identification of CD 20 expression in the tumour cells, rituximab can be used in the treatment of that specific leukemia and lymphoma. Similarly, co-expression of myeloid antigens in B Cell acute lymphoblastic leukemia may indicate presence of Philadelphia chromosome which is a poor prognostic marker. Absence of CD 10 is associated with MLL translocation. MLL translocation is a negative prognostic marker for infants with ALL.

iii. **Minimal residual disease (MRD) assessment in acute leukemia: (MRD)** is a term used to indicate the presence of small number of malignant cells in the body after chemotherapy. Traditionally, response to therapy in leukemia is done by assessing the percentage of blast cells in bone marrow aspiration at the end of induction. Pathologists interpret this marrow by looking for blasts in at least 200 cells

microscopically. Children normally have hypercellular marrow and high number of recovering normal immature cells (hematogones) post chemotherapy which resemble blasts. It is difficult to differentiate hematogones from leukemic blasts morphologically in a bone marrow aspirate.

Flow cytometric assessment of minimal residual disease (MRD) at the end of induction utilizes CD 19, 10 and 22 co-expression to differentiate leukemia cells from hematogones. This leukemia associated immuno phenotype (LAIP) identification happens by analysing 2,00,000 events and it is reported as % of cells expressing LAIP. For B cell acute lymphoblastic leukemia, MRD should be <0.01% and for T Cell acute lymphoblastic leukemia, < 0.1% at end of consolidation. MRD is an important prognostic marker in ALL therapy and indicates chemo responsiveness of the tumors.[5]

iv. Complementing cytology in the diagnosis of central nervous system leukemia

v. **Pediatric solid tumours:** In neuroblastoma, cells from bone marrow or peripheral blood show positivity for CD44, CD56, CD81, CD9 and CD 45 negative and malignant cells in Ewings sarcoma are positive for CDD 99 and negative for CD 45.[5]

B. Primary immune deficiency (PID) work up

In PID, flow cytometry has been much useful for the diagnosis and monitoring of the following diseases

i. **Diseases with quantitative defects in T Cells and B cells**: It helps in rapid diagnosis of quantitative deficiencies of T and B cell disorders. For eg. in severe combined immune deficiency, the absolute CD 3 is often less than 300 cells/ microL in peripheral blood with or without B cell and NK cell deficiencies[6,7] Similarly, in Bruton's Agammaglobulinemia, absolute B cell numbers (CD19) are less or absent along with hypogammaglobulinemia. Autoimmune lymphoproliferative syndrome (ALPS) is characterised by generalized lymphadenopathy, splenomegaly, autoimmunity along with increased infections which is diagnosed by elevated number of double negative T Cells (CD3+TCR$\alpha\beta$+CD4-CD8- DNT).[3]

ii. **CD 4 counts monitoring in HIV/ AIDS:** Flow cytometry is also used for serial CD4 counts measurement in monitoring of Acquired Immune Deficiency Syndrome (AIDS) therapy.[8]

iii. **Neutrophil defects: Dihydro Rhodamine (**DHR) assay by flow cytometry provides an objective measure of neutrophil function as against the subjective Nitroblue Tetrazolium Test (NBT) assay. Quantitative assessment of CD 11 and 18 by flow cytometry is used for diagnosis of leukocyte adhesion deficiency.[3]

C. Stem cell marker and bone marrow transplantation (BMT)

Flow cytometry is utilised for the following indications in bone marrow transplantation

i. **Quantification of CD 34 and cell viability assessment in stem cell product:** CD 34 is used as a marker of stem cell in the bone marrow or peripheral blood stem cell harvest product. For a successful bone marrow transplant, we require a minimum of 2x 10 ^{6}/kg (of recipient's weight) of CD34 stem cells. Further, flow cytometry can also assess cell viability which is important in stem cell products which are cryopreserved. Post thawing, viability of cells is assessed to quantify meaningful cell dose in cryopreserved products during bone marrow transplant.

ii. **Ex-vivo T cell depletion for haploidentical bone marrow transplantation:** HLA barrier in haploidentical bone marrow transplants has been overcome with T- Cell depletion. With flowcytometric enumeration of T Cell subsets (CD3/ CD4/CD8 / T Cell Receptor Alpha Beta/ T Cell Receptor Gamma Delta), B cells and CD34, it is feasible to manipulate the stem cell product and provide high stem cell dose (CD 34 positive selection) with T cell (TCR $\alpha\beta$) and B cell depletion.[9]

D. Benign hematological illnesses

Flow cytometry is useful in the diagnosis of following hematological illnesses

i. **Paroxysmal Nocturnal Hemoglobinuria (PNH):** This acquired hemolytic anemia due to complement defects is diagnosed by flow cytometric assessment of CD 55 and CD59. This diagnostic test has replaced the conventional tests like Ham's test and Sucrose lysis test.[4]

ii. **Hereditary spherocytosis (HS):** Presence of family history, spherocytosis and a negative Coomb's test in a child with hemolytic anemia is sufficient for diagnosis of HS. In patients with absence of family history, and to diagnose HS in the new born period, eosin-5-maleimide (EMA) study by flowcytometry to assess band 3 in red cell membrane is a useful diagnostic test.[10]

iii. **Fetal maternal hemorrhage:** In fetal maternal hemorrhage, fetal red cells in maternal blood can be demonstrated by the traditional Kleihauer Betke Test based on the concept of fetal red cells being resistant to alkali denaturation. This test, to some extent, also helps in quantification of the fetal red cells. Kleihauer Betke test is known to be tedious, time-consuming and shows high inter-observer variability.[4,11] Flowcytometry can also be used to assess the fetal RBC by identifying fluorescent labelled antibodies against fetal haemoglobin or RBCs with Rh Antigen circulating in maternal blood.

III. Limitations of flow cytometry[1,5]

1. The cells need to be suspended in fluid which limits flowcytometric testing to organs like blood, bone marrow and lymph nodes where cells can be easily dissociated.

2. Difficulty in standardization of antigens markers, fluorescence, antibody conjugates, instrumentation and software analysis.

IV. Case scenarios

Case 1

Eight months old male child was brought with complaints of failure to thrive and recurrent respiratory infections since 5 months of age. He was born of second-degree consanguineous marriage and his elder female sibling, 4 years old, was thriving well. His birth weight was 3 kg and his weight increased till 4 months of age and subsequently remained static at 6 kg. On examination, he had oral candidiasis along with left axillary lymphadenopathy, he had no dysmorphic features; BCG scar showed mild inflammation; he had moderate hepatosplenomegaly. Auscultation of the chest showed bilateral crepitations and chest x ray showed reticulo-nodular pattern. He was negative for HIV ELISA serology. Blood investigations revealed low hemoglobin (6g/dL), low WBC count (4500/ microL) with polymorphs of 75% lymphocytes of 22% and eosinophils of 3% and normal platelet count (4.2 Lakhs/ microL). His absolute neutrophil count was 3375/ cu.mm and absolute lymphocyte count was 990/ cu.mm.

With onset of serious infections in 1st 6 months of age, along with occurrence of oral candidiasis, delayed healing of BCG site and low absolute lymphocyte count, possibility of severe combined immune deficiency was considered. His serum immunoglobulin profile revealed low IgG (140 mg/dL Range 350 - 1620 mg/ dL) low IgA (< 25mg/dL, normal : 17 - 318 mg/dL) and low IgM levels (22 mg/dL, normal: 40 - 230 mg/dL) . Lymphocyte subset analysis is shown in Table III.

Table III. Lymphocyte subset analysis of case 1

Cells (units)	Value	Normal range
Total WBC count (/cu.mm)	4500	4000 to 11000
Lymphocytes (%)	**22**	
Absolute Lymphocyte Count	**990**	1900 – 3700
Lymphocyte Subsets		
B Lymphocytes CD19+ (%)	**2**	6 – 23
Absolute B Lymphocytes (/cu.mm)	**90**	270 – 860
T Lymphocytes CD 3+ (%)	**4**	62 – 87
Absolute T Lymphocytes (/cu.mm)	**180**	570 – 2400
Helper T Cells Th Lymphocytes CD3+/CD4+ (%)	**3**	32 – 64
Absolute Th Lymphocytes (/cu.mm)	**135**	650 – 1500
Cytotoxic T Cells Tc Lymphocytes CD 3+/CD8+ (%)	**1**	15 – 46
Absolute Tc Lymphocytes (/cu.mm)	**45**	370 – 1100
NK Cell CD16+/CD56+ (%)	15	4 – 26
Absolute NK Cell (/cu.mm)	675	100 – 480

Diagnosis was T – B – NK + SCID with mutation studies revealing RAG 1 defect.

Case 2

Four years old girl presented with painful joint swelling involving both knee joints and right elbow joint, non-migratory pain, for last 2 months. She had history of anemia (Hb - 4 gram/dL) requiring blood transfusion 1 month back. Her examination was unremarkable with no enlarged nodes or organomegaly. With possibility of juvenile idiopathic arthritis (JIA), she was further evaluated. Her blood investigations revealed anemia (Hb - 7.4 g/dL), normal WBC count (9800/ cu.mm), relative lymphocytosis (polymorphs 35% lymphocytes 65%) and normal platelet count (3.75lakhs/cu.mm). Mean Corpuscular volume (MCV) was high (95 fl). Her biochemical parameters were within normal limits. ESR was high 120 mm/hour with positive CRP (12 mg/dL).

Table IV. Lymphocyte subset analysis of case 2

Marker	% positivity	Interpretation
T cell markers		
CD3	0.3	Negative
Cyt CD3	4.3	Negative
CD5	0.3	Negative
CD7	0.8	Negative
B cell markers		
CD 19	**98.8**	**Positive**
CD10	**91.3**	**Positive**
CD22	**92.6**	**Positive**
CD 20	**38.0**	**Positive**
S IgM	0	Negative
Myeloid markers		
CD13	3.0	Negative
CD14	0	Negative
CD16	0.1	Negative
CD11b	1.2	Negative
CD33	0	Negative
CD64	0	Negative
MPO	0	Negative
Others		
CD34	13.1	Negative
CD45	**100**	**Positive**
CD117	0	Negative
HLADR	**98.2**	**Positive**
TdT	0.6	Negative
CD36	1.6	Negative
CD38	**98.1**	**Positive**
CD56	0.1	Negative

Bone marrow evaluation was done to rule out hematological malignancies prior to start of steroid therapy for JIA. Bone marrow aspiration showed 25 - 30% atypical cells with suspicion of blasts. Flow cytometry for acute leukemia panel is given in Table IV.

Flow cytometry cell preparation method: Stain-Lyse-Wash

Flow cytometric analysis reveals a cell cluster in CD 45 Dim region with low side scatter. These gated cells in blast region (35%) shows moderate expression of CD 19, CD 10, HLADR, dim expression of CD22, CD 20, partial dim expression of CD34, dim to moderate expression of CD 38 - suggestive of B cell acute lymphoblastic leukemia (B - ALL).

Child's final diagnosis was B cell acute lymphoblastic leukemia. Arthritic presentation of ALL can masquerade as JIA.

Conclusion

Flow cytometry helps in analysing multiple characteristics of complex cells in a short period of time. Flowcytometer analyses cells suspended in liquid media and sorts them based on cell size and cellular content when it passes through a light source. Fluorescent labelled antibodies emit light and helps in further categorization of cells based on cellular and cytoplasmic CD Antigens. It can categorize cells by its size, cytoplasmic contents, DNA, RNA content and various membrane and cytoplasmic protein.

The indications and uses of flow cytometry are increasing with time. Currently, flow cytometry is used to diagnose, stratify risk and treat hematological malignancies. Other than leukemias and lymphomas, flow cytometry is also being explored in pediatric solid tumours like neuroblastoma, wherein cells from bone marrow or peripheral blood will show positivity for CD44, CD56, CD81, CD9 and be negative for CD 45 and Ewings sarcoma in which malignant cells are positive for CDD 99 and negative for CD 45.[5]

Points to Remember

- ***Flow cytometry analyses various qualitative and quantitative characteristics of a cell, such as cell size and cellular contents.***
- ***Though the mechanism is complex, it has wide application in the diagnosis of various hematological conditions ranging from benign disorders like fetal maternal hemorrhage to malignancy and immune deficiency.***
- ***Identification odf minimal residual diseases plays a major role in the management of children with leukemia.***
- ***Flow cytometry also helps in identifying prognostic markers and markers for therapeutic use, such as use of rituximab in tumor cells expressing CD 20.***

References

1. Woo J, Baumann A and Arguello V. Recent advancements of flow cytometry: new applications in hematology and oncology. Expert Rev Mol Diagn 2014; 14(1):67-81.
2. Engel P, Boumsell L, Balderas R, Bensussan A, Gattei V, Horejsi V, et al. CD Nomenclature 2015: Human Leukocyte Differentiation Antigen Workshops as a Driving Force in Immunology. J Immunol 2015; 195:4555-4563.
3. Bonilla FA, Notarangelo L. Primary Immunodeficiency Diseases. In: Nathan and Oski's Hematology of Infancy and Childhood. 8th edn. Philadelphia: Elsevier Saunders, 2015: 886- 921.
4. Brown M, Wittwer C. Flow Cytometry: Principles and Clinical Applications in Hematology. Clinical Chemistry 2000; 46:1221-1229.
5. Varma N, Naseem S. Application of Flow Cytometry in Pediatric Hematology-Oncology. Pediatr Blood Cancer 2011; 57:18-29.
6. de Vries E, Noordzij JG, Kuijpers TW, van Dongen JJ. Flow cytometric immunophenotyping in the diagnosis and follow-up of immunodeficient children. Eur J Pediatr 2001; 160:583-591.
7. Warren JS. Primary immunodeficiency diseases. In: McClatchey KD, ed. Clinical Laboratory Medicine. 2nd ed. Philadelphia: Lippincott, Williams, and Wilkins, 2002; pp1458-1470.
8. Hengel RL, Nicholson JKA. An update on the use of flow cytometry in HIV infection and AIDS. Clin Lab Med 2001; 21:841-856.
9. Lamb LS. Hematopoietic cellular therapy: Implications for the flow cytometry laboratory. Hematol Oncol Clin North Am 2002;16:455-476.
10. Fanhchaksai K, Manowong S, Natesirinilkul R, Sathitsamitphong L, Charoenkwan P, et al. Flow Cytometric Test with Eosin-5-Maleimide for a Diagnosis of Hereditary Spherocytosis in a Newborn. Case Reports in Hematology 2019: Article ID 5925731. (https://doi.org/10.1155/2019/5925731).
11. Davis BH, Olsen S, Bigelow NC, Chen JC. Detection of fetal red cells in fetomaternal hemorrhage using a fetal hemoglobin monoclonal antibody by flow cytometry. Transfusion 1998; 38:749-756.

CLIPPINGS

Self-amplifying RNA vaccines.

Vaccinology is shifting toward synthetic RNA platforms which allow for rapid, scalable, and cell-free manufacturing of prophylactic and therapeutic vaccines. The simple development pipeline is based on in vitro transcription of antigen encoding sequences or immunotherapies as synthetic RNA transcripts, which are then formulated for delivery. This approach may enable a quicker response to emerging disease outbreaks, as is evident from the swift pursuit of RNA vaccine candidates for the global SARS-CoV-2 pandemic. Both conventional and self-amplifying RNAs have shown protective immunization in preclinical studies against multiple infectious diseases including influenza, RSV, Rabies, Ebola and HIV-1. Self-amplifying RNAs have shown enhanced antigen expression at lower doses compared to conventional mRNA.

Bloom K, Van den Berg F, Arbuthnot P. Self-amplifying RNA vaccines for infectious diseases. Gene Ther. https://doi.org/10.1038/s41434-020-00204-ySpringer Nature Limited 2020.

ADOLESCENCE

MANAGEMENT OF ADOLESCENT SUICIDAL BEHAVIOUR

**** Amitha Rao Aroor***
***** Preeti M Galagali***

Abstract: *Suicide is one of the leading causes of adolescent mortality globally and in India. Genetic susceptibility, underlying psychiatric illness and negative life events make vulnerable adolescents take this drastic step. Questions about suicidal ideation should be asked during routine HEEADSSS assessment in non-judgmental manner and those with suicidal ideation should be asked about the intent and plan and need detailed evaluation for risk stratification. In addition to screening and detailed evaluation, initial counselling should be done by the pediatrician and consultation with a mental health specialist must be arranged on an emergency basis.*

Keywords: *Suicide, Adolescents, Risk stratification, Safety planning, Prevention.*

Suicide among adolescents has emerged as a significant global health problem. As per WHO global health estimates it is the second leading cause of death among young people aged 15-29 years, third leading cause of death in 15-19 year olds for both sexes and the top cause of adolescent mortality in India.[1] According to Lancet 2012 report, India has one of the highest suicide rates for the youth aged 15-29 years.[2] Because of the stigma attached to mental health care, pediatricians may be the first point of contact and hence play a crucial role in identifying and managing these at risk adolescents. This article focuses on screening and office management of adolescent suicidal behaviour.

Vulnerability of adolescents to suicide

Adolescence is the period of heightened emotional reactivity. Early development of limbic system or emotional brain compared to the prefrontal cortex or reasoning brain (responsible for impulse control, decision making, executive functions) which does not fully mature until mid-twenties makes them more vulnerable to suicidal attempts. In addition, adolescent brain is highly sensitive to various stressors and media influence. Teens are more susceptible to suicide contagion i.e. a process by which direct or indirect exposure to suicide of one or more persons influence others to attempt suicide. Vulnerable youth are more susceptible to the reports of suicide in mass media. Evidences of influence of media on suicidal behaviour has been shown for newspaper and television reports of actual suicides, film and television portrayals of suicide and for suicide manuals.[3] Social media can also have negative impact due to cyber bullying, sexting and providing information about self-harm techniques.

Terminologies: Terminologies related to adolescent suicide are as in Table I.[4]

Factors associated with adolescent suicide: Suicide is the end result of a complex interplay between genetic, psychiatric and various other factors. Predisposing biological (serotonin imbalance), personality (perfectionism, impulsivity) and cognitive vulnerabilities (impaired problem solving) combine with negative life events and psychiatric disorders and increase the risk of suicide (Fig.1).[5]

Risk and protective factors: There are multiple risk factors for adolescent suicide. All adolescents with suicidal

Table I. Terminologies related to adolescent suicide

Suicidal ideation (SI)	Consideration of or desire to end one's own life. Can range from passive SI (wanting to be dead) to active SI (wanting to kill oneself)
Suicidal plans	Thoughts related to designing and engaging in the act of suicide
Suicide attempt	An action intended to deliberately end one's own life.

* Professor in Pediatrics and Adolescent Health Specialist, A.J Institute of Medical Sciences, Mangalore, Karnataka.

** Director and Adolescent Health Specialist, Bengaluru Adolescent Care and Counseling Centre, Bengaluru, Karnataka.

email: amithaaroor@gmail.com

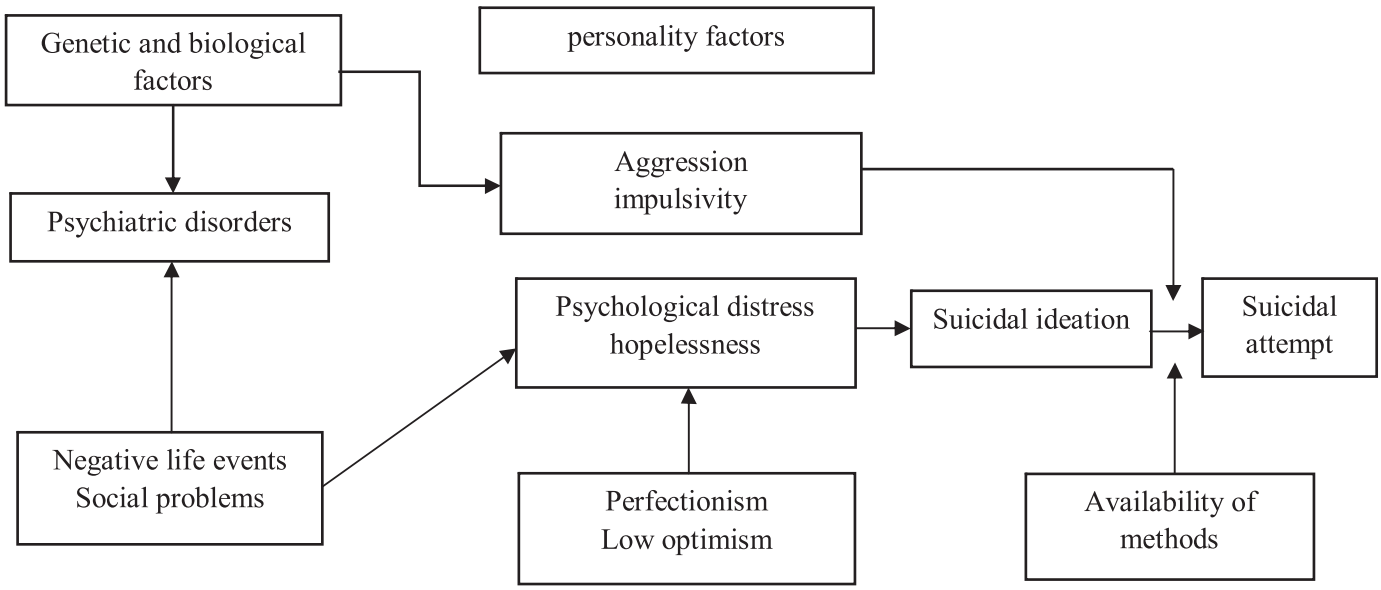


Fig.1. Key factors in adolescent suicide

ideation should be screened for underlying psychiatric illness. Previous self-harm or suicidal attempts increase the suicidal risk. It indicates that the person is capable of moving from suicidal thoughts to behaviour. On the other hand, protective factors promote resilience and reduce the potential for suicide. Important risk and protective factors are shown in Table II.[5,6,7]

Role of pediatrician in management of a suicidal adolescent: Table III highlights the role of a pediatrician in office management of a suicidal adolescent.

Clinical assessment of suicidal risk: Adolescents usually do not reveal suicidal thoughts unless asked. HEEADSSS assessment should be done in all adolescents to explore into psychosocial issues beyond the presenting complaints.[8] Questions related to suicide should be asked at the end of HEEADSSS screening (HEEADSSS is a useful tool and an acronym for psychosocial screening of adolescents: Home, Education & Employment, Eating, Activities, Drugs/Drinking, Sexuality, Suicide/depression, Safety).

Establishing rapport, active listening, being non-judgmental and demonstrating empathy are important during the assessment. Research shows that asking youth about suicidal thoughts does not increase risk of suicide, rather it makes them more comfortable to discuss when they are distressed.[6] The American Academy of Pediatrics recommends routine screening of adolescents about depression, suicidal thoughts and other risk factors associated with suicide.[7] In addition to interviewing the adolescent alone, collateral information should be obtained from parents and other gatekeepers.

Confidentiality: Parents need to be informed when there is suicidal risk and also the adolescent needs to be told about the necessity to break confidentiality as safety takes precedence over confidentiality.[7]

Assessing current suicidality: When asking about suicidal thoughts the questions must be open-ended as much as possible. Normalising the statements when introducing the topic leads to more open disclosure. Suicidal ideation, intent and plan have to be assessed.[9] Asking about suicide ideation is to identify youth at risk for suicide by asking about the suicidal thoughts and their frequency as well as intensity. The presence of suicidal intent should be assessed in all those having ideation. Acts of self-harm might or might not be associated with true intent to commit suicide. They need to be specifically asked whether the self-harm behaviour was intended to relieve psychological pain (non suicidal self injury) (NSSI) or whether there was intention to commit suicide. Details of the plan if present should be asked. Sample questions for suicidal ideation, intent and plan are depicted in Table IV.

If there is previous suicide attempt, the following details should be asked.[9]

- The method used (unusual methods carry higher risk)
- Whether it was impulsive or planned
- The lethality of the method used
- The anticipated outcome
- Steps taken to decrease the likelihood of being discovered (signifies higher intent)

Table II. Important risk and protective factors in adolescent suicide

Risk factors	Protective factors
Family history of suicide	Positive connections to school
Mental health issues in parents	Coping, problem-solving and emotion regulation skills
Parental death/divorce	
LGBTQ community	Academic achievement
Adverse childhood experiences	Family stability
Physical /sexual abuse	Help-seeking behaviour
Bullying	
Impaired parent-child relationship	Good peer relationships
Poor family communication	Positive self-worth
Low parental monitoring	Impulse control-conflict resolution abilities
Living outside of the home	Access to care for mental/ physical/substance disorders
Difficulties in school	Lack of access to means of suicide
Presence of stressful life events	
Psychiatric / psychological factors:	
- Depression	
- Anxiety	
- ADHD	
- Substance abuse	
- Pathologic internet use	
- Low self esteem	
- Impulsivity	
- Hopelessness	

LGBTQ - lesbian, gay, bisexual, transgender, queer

Examination: Detailed clinical examination should be done including assessment of

- General appearance (dressing, eye contact, grooming, facial expression, etc.)
- Attitude (cooperative/violent/avoidant)
- Activity (calm/restless)

Table III. Pediatrician's role in office management of suicidal adolescent

1. Screening
2. Evaluation of risk and protective factors, risk stratification
3. Brief intervention- immediate counseling with reassurance
4. Safety planning
5. Referral
6. Documentation
7. Follow up
8. Prevention

- Detailed mental status examination
- Trauma findings [abuse, substance abuse, hesitation cuts (self-inflicted superficial cuts made by those considering suicidal attempt)]
- Family interactions
- Evidence of systemic diseases
- Signs of depression, substance use, psychosis and other mental health illness

Warning signs of suicide: A suicide warning sign indicates heightened risk for suicide in the near-future (i.e., within minutes, hours, or days).[10] Most adolescents will exhibit warning signs of suicide which need to be identified. American Association of Suicidology has developed the mnemonic 'IS PATH WARM' to identify warning signs of suicide[6,11,12] (Table V).

Risk stratification: Assessing suicide risk level can be difficult because of multiple factors associated with the clinical presentation. Assessment needs evaluation of suicidal thoughts, level of intent, existence of plan, access to means, stressors, family and social support, etc. All suicidal ideation and attempts should be taken seriously and requires assessment by mental health specialist.

- Emergency mental health assessment is needed for immediate threat to life (suicidal intent and plan).
- Urgent mental health assessment (48-72 hours) is needed for severe psychiatric symptoms, significant change in overall functioning and/or suicidal ideation without intent or plan.
- Routine mental health assessment is required for mild to moderate psychiatric symptoms without suicidal ideation.[13]

Table IV. Interviewing for suicidal ideation, intent and plan

Ideation	Intent	Plan
- Sometimes people think about hurting or killing themselves when they are upset. Have you anytime experienced such thoughts?	- Have you ever thought of acting on your thoughts?	- Do you have a plan? If so, how are you planning to do it?
- How often do you get these thoughts and how long do they last?	- How likely do you think you are to carry out your plan?	- Do you have means that you would use?
- How difficult is it for you to distract yourself when you have such thought?		- Is there something that would trigger the plan?
- What do you do when you have such thoughts? What coping strategies do you use?		
- What is the worst they have ever been? What did you do?		
- Are there any triggering events?		
- Do you have hope that things will get better (significant hopelessness has increased suicidal risk)		

Table V. Warning signs of suicide

I	Ideation	Talking / writing about death, threatening to hurt or kill self, looking for ways to die, behaviors or statements indicating good-byes, including giving away prized possessions
S	Substance Use	Increased substance use
P	Purposelessness	Having no reason to live; neglecting appearance and hygiene
A	Anxiety(worry/fear)	Anxiety, agitation, unable to sleep
T	Trapped	Feeling like there is no way out of a bad situation, believing suicide is the only solution to one's problems
H	Hopelessness	Hopelessness about future
W	Withdrawal	From friends/family/society
A	Anger	Rage, uncontrolled anger, seeking revenge
R	Recklessness	Engaging in risk activities, not caring for the consequences
M	Mood changes	Suddenly improving following a severe depression; dramatic mood changes

A simple method suggested to save lives from suicide is QPR. QPR refers to Question, Persuade and Refer. Exploring suicidal thoughts, persuading to seek help and making timely referral can prevent teens from taking the drastic step. Risk is high in patients who report active suicide ideation with specific plan or intent and have access to lethal means. The clearer the content, higher the risk. Passive ideation should not be ignored as it can progress to active ideation with plan. The management plan depends on the risk categorization as shown below in Fig.2.[13]

Risk categorisation in patients with previous suicidal attempt / Non-suicidal Self-Injury (NSSI): When determining the risk, it is essential to assess chronic and acute risk level. Chronic risk level is determined by history of suicidal/non suicidal injury behaviours in the past. Risk stratification based on chronic and acute risk is shown in Fig.3.[14]

Safety planning: Implementation of safety planning provides strategies to prevent and manage suicidal crisis. Safety planning intervention (SPI) developed by Stanley

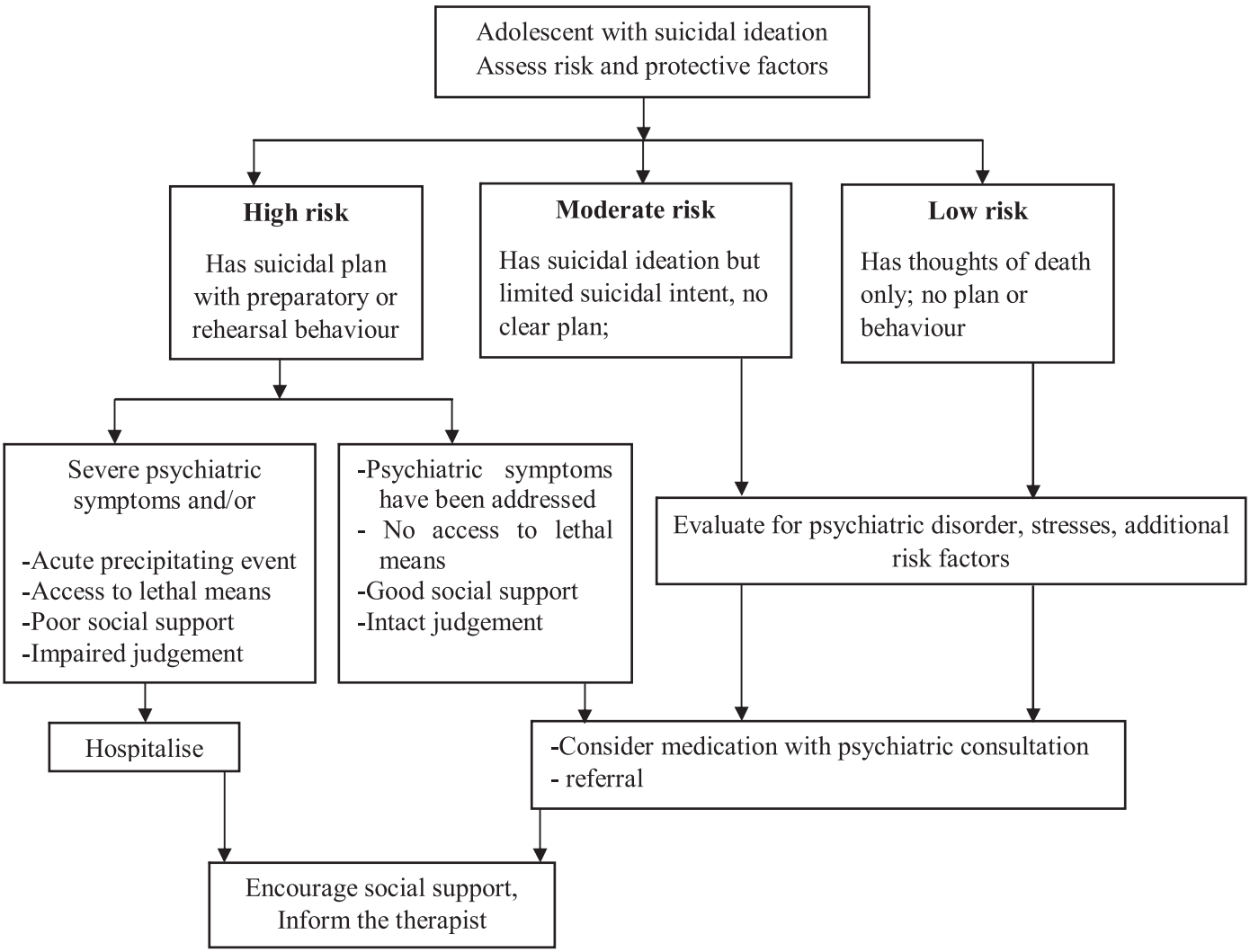


Fig.2. Risk categorisation and management plan for an adolescent with suicidal behaviour

and Brown is easy to implement and has been found to be effective in preliminary studies.[14,15] The safety plan should be given to the adolescent and caregivers to use when they experience crisis.

Rationale of SPI: Suicidal crisis consists of a short period of urges to end life. Preventing youth from acting on these urges allows time for crisis to dissipate.

It consists of 6 steps to help individuals recognize signs of impending suicidal crisis and utilise coping strategies and resources to prevent suicide attempt. Also needs involvement of caregivers in implementing the plan.

Step1: Recognizing warning signs of impending suicidal crisis

Step2: Employing internal coping strategies to distract themselves in a positive way.

Step 3: Utilizing social contacts (people with whom they share positive relationships) and social settings (mall, park, etc.) as means of distraction.

Step 4: Utilizing trusted adults to help resolve the crisis.

Step 5: Contacting health professionals/helpline.

Step 6: Making environment safe and limit access to lethal means.

Screening Tools: Standardized screening instruments for depression and suicidal ideation are available. They may complement but should never replace thorough assessment. Ask Suicide Screening Questionnaire (ASQ) is a validated 4 item measure with good sensitivity and negative predictive value in identifying youth at risk for suicide.[13,16] Others include Columbia Suicide Screen,[17] Suicidal Ideation Questionnaire,[18] Beck Depression Inventory,[19,20] Patient Health Questionnaire 9.[21]

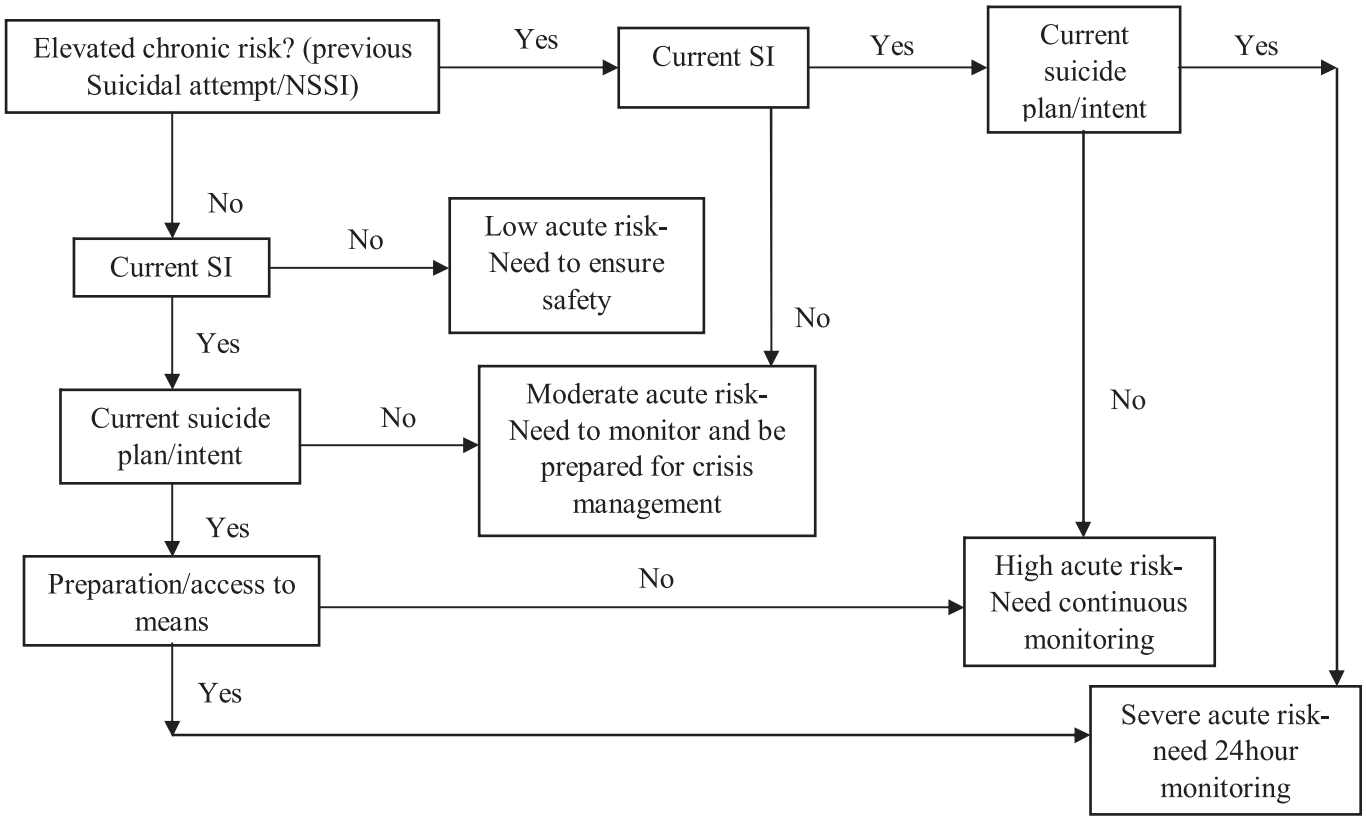


Fig.3. Risk stratification in patients with previous suicidal attempt/NSSI

Medicolegal implications and documentation: All the interactions with suicidal adolescents should be clearly documented to further help patient and for legal purposes.

Documentation should include:

- Summary of presenting complaints
- Evaluation of current risk/ protective factors, warning signs, clinical formulation of suicide risk
- Acute and chronic risk stratification
- List of individuals who participated in the evaluation
- Summary of treatment options discussed including recommendation for hospitalization, referrals, completed safety plan
- Follow-up plan

Prevention of adolescent suicide: Many of the teen suicides are preventable and it requires combined effort and education of the adolescent and the gate keepers. Suicide prevention can be primary prevention (to prevent the onset of suicidal thoughts) which include promoting resilience, promoting peer/ family connectedness, helping teens overcome distress by using coping strategies and secondary prevention to detect youth at risk of suicide and recognize those with warning signs.[22] Some of the measures to reduce teen suicide are as follows.

1.Crisis care: For those with suicidal ideation and /or with previous attempt

2.Treatment of mental illness: By psychotherapy (e.g. - cognitive behavioural therapy) and pharmacotherapy. Those who are on selective serotonin reuptake inhibitor (SSRIs) need to be monitored for suicidal ideation during the initial weeks of therapy.

3.Training of pediatricians: Pediatricians play an important role in suicide prevention by identifying behavioural and emotional problems, promoting positive parenting skills and by promoting resilience among youth and families.

4.Gatekeeper (Adults who regularly interact with adolescents and who may recognise problems and help) Training: To identify at risk behaviour and promote resilience.

5.School programs: Focused on life skill education, knowledge about warning signs and promote help seeking.

6.Community programs: To promote mental health and reduce stigma associated with mental illness.

7.Role of media: Responsible reporting of suicide by media, detecting youth with suicide risk with their social media posts, running youth awareness campaigns on social media and offering online consultations

8.Means restriction: Restricting the access to common means of suicide.

Points to Remember

- ***Adolescence is the period with high vulnerability to various high risk behaviours. Suicide is one of the top causes of adolescent mortality in India and is the result of interplay of genetic and multiple environmental factors.***
- ***Adolescents do not reveal suicidal thoughts unless asked and hence screening for psychosocial issues should be performed in all of them beyond the presenting complaints.***
- ***Assessment of suicidal behavior includes current suicidal ideation, intent, plan, past attempts and assessment of risk as well as protective factors.***
- ***Many of the adolescents exhibit one or more of the warning signs which need to be recognized by the caretakers. Any suicidal threat should be taken seriously.***
- ***Screening tools should only supplement and not replace thorough clinical evaluation***
- ***Management depends on the risk stratification and referral to mental health specialist is a must in all those with suicidal ideation.***
- ***Safety planning intervention should be given to the at-risk adolescent who should be educated to use the same during crisis.***
- ***Many suicidal attempts are preventable with adequate training of 'the gate keepers'.***

References

1. World Health Organization. Suicide in the world: Global health estimates. 2019. Available at: https://apps.who.int/iris/handle/10665/326948. License: CC BY-NC-SA 3.0 IGO. Accessed on:23 August 2019.
2. Patel V, Ramasundarahettige C, Vijayakumar L, Thakur JS, Gajalakshmi V, Gururaj G, et al. Suicide mortality in India: a nationally representative survey. Lancet 2012;379(9834):2343-2351.
3. Hawton K, Williams K. Influences of the media on suicide. BMJ 2002;325(7377):1374-1375.
4. Cha CB, Franz PJ, Guzmán ME, Glenn CR, Kleiman EM, Nock MK. Annual Research Review: Suicide among youth - epidemiology, (potential) etiology, and treatment. Child Psychol Psychiatry 2018;59(4):460-482.
5. Hawton K, Saunders KE, O'Connor RC. Self harm and suicide in adolescents. Lancet 2012; 379(9834):2373-2382.
6. Taliaferro LA, Oberstar JV, Borowsky IW. Prevention of youth suicide: The role of the primary care physician. Journal of Clinical Outcomes Management2012; 19 (6):270-285.
7. Shain B and AAP Committee on Adolescence. Suicide and Suicide Attempts in Adolescents. Pediatrics 2016;138(1): e20161420.
8. KleinDA, Goldenring JM, Adelman WP. HEEADSSS 3.0: The psychosocial interview for adolescents updated for a new century fueled by media. Contemp Pediatr; 2014: 31(1):16-28.
9. Daphne J Korczak, Canadian Pediatric Society, Mental Health and Developmental Disabilities Committee. Suicidal ideation and behavior. Pediatr Child Health 2015; 20(5):257-260.
10. Rudd MD, Berman AL, Joiner TE Jr, Nock MK, Silverman MM, Michael M, et al. Warning Signs for Suicide: Theory, Research, and Clinical Applications. Suicide Life Threat Behav 2006; 36(3):255-262.
11. Dilillo D, Mauri S, Mantegazza C, Fabiano V, Mameli C, Zuccotti GV. Suicide in pediatrics: epidemiology, risk factors, warning signs and the role of the pediatrician in detecting them. Ital J Pediatr 2015; 41(1):1-8.
12. Juhnke GA, Granello PF, Lebrón-Striker MA. IS PATH WARM? A suicide assessment mnemonic for counselors (ACAPCD-03). Alexandria, VA: American Counseling Association, 2007.
13. Demaso DR, Walter HJ, Wharff EA. Suicide and attempted suicide. Nelson textbook of Paediatrics,21st edition, Philadelphia: Elsevier; 2019; pp159-162.
14. Pettit JW, Buitron V, Green KL. Assessment and Management of Suicide Risk in Children and Adolescents. Cogn Behav Pract 2018; 25(4):460-472.
15. Stanley B,Brown GK. Safety planning intervention: A brief intervention to mitigate suicide risk. Cogn Behav Pract 2012; 19(2):256-264.
16. Horowitz LM, Bridge JA, Teach SJ, Ballard E, Klima J, Rosenstein DL, et al. Ask Suicide-Screening Questions (ASQ): a brief instrument for the pediatric emergency department. Arch Pediatr Adolesc Med 2012;166 (12):1170-1176.
17. Shaffer D, Scott M, Wilcox H, Maslow C, Hicks R, Lucas CP, et al. The Columbia Suicide Screen: validity and reliability of a screen for youth suicide and depression. J Am Acad Child Adolesc Psychiatry 2004; 43(1):71-79.
18. Keane EM, Dick RW, Bechtold DW, Manson SM. Predictive and concurrent validity of the Suicidal Ideation Questionnaire among American Indian adolescents. J Abnorm Child Psychol 1996; 24(6):735-747.

19. Gordon Jackson-Koku. Beck Depression Inventory. Occup Med 2016; 66(2):174-175.
20. Winter LB, Steer RA, Jones-Hicks L, Beck AT. Screening for major depression disorders in adolescent medical outpatients with the Beck Depression Inventory for Primary Care. J Adolesc Health 1999;24(6): 389-394.
21. Richardson LP, McCauley E, Grossman DC, McCarty CA, Richards J, Russo JE, et al. Evaluation of the Patient Health Questionnaire-9 item for detecting major depression among adolescents. Pediatrics 2010; 126(6):1117-1123.
22. Horowitz L, TiptonMV, PaoM. Primary and secondary prevention of youth suicide. Pediatrics 2020;145(s2): S195-203.

CLIPPINGS

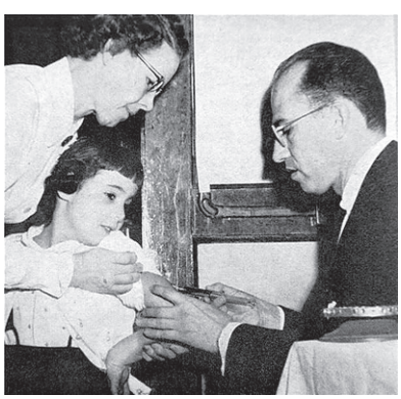
Nonhuman primate to human immunobridging to infer the protective effect of an Ebola virus vaccine candidate

It has been a huge challenge to conduct traditional efficacy trials for Ebola virus (EBOV) vaccines. In the absence of efficacy data, immunobridging is an approach to infer the likelihood of a vaccine protective effect, by translating vaccine immunogenicity in humans to a protective effect, using the relationship between vaccine immunogenicity and the desired outcome in a suitable animal model. For EBOV disease (EVD), nonhuman primates (NHP) are the most relevant animal model, exhibiting the major hallmarks of hemorrhagic fever such as clotting abnormalities as well as liver and kidney damage, albeit with differences in disease course and severity associated with a higher lethality rate. In this case, cynomolgus monkeys (Macaca fascicularis) are used, in which EVD appears stringent, even when compared to the most severe human cases.

The authors propose to infer the protective effect of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen with an 8-week interval in humans by immunobridging. Immunogenicity and protective efficacy data were obtained for Ad26.ZEBOV and MVA-BN-Filo vaccine regimens using a fully lethal EBOV Kikwit challenge model in cynomolgus monkeys (nonhuman primates [NHP]). The association between EBOV neutralizing antibodies, glycoprotein (GP)-binding antibodies and GP-reactive T cells and survival in NHP was assessed by logistic regression analysis.

Binding antibodies against the EBOV surface GP were identified as the immune parameter with the strongest correlation to survival post EBOV challenge, and used to infer the predicted protective effect of the vaccine in humans using published data from phase I studies. The human vaccine-elicited EBOV GP-binding antibody levels were in a range associated with significant protection against mortality in NHP. Based on this immunobridging analysis, the EBOV GP-specific-binding antibody levels elicited by the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen in humans will likely provide protection against EBOV disease.

Roozendaal R, Hendriks J, van Effelterre T, Spiessens B, Dekking L, Solforosi L, et al. Nonhuman primate to human immunobridging to infer the protective effect of an Ebola virus vaccine candidate. npj Vaccines 5, 112 (2020). https://doi.org/10.1038/s41541-020-00261-9.



Jonas Salk administering Polio vaccine

Magazine photo of Jonas Salk (Polio vaccine discoverer) to O'Neill, during the polio vaccine field trial (1954) involving 20,000 physicians and public health officers, 64,000 school personnel and 220,000 volunteers, with over 1.8 million school children, including his three sons participating in the trial.

Source: Solanki RS, Mehendale AM A tribute to Jonas Salk: A journey towards polio free world. Journal of Mahatma Gandhi Institute of Medical Sciences 2016; 21: (2) 89-91.

RADIOLOGY

CYSTIC LESIONS IN NEONATES AND INFANTS ON CRANIAL ULTRASONOGRAM

** Raveendran J*
*** Balaji S*

Ultrasound of cranium in neonates and children often shows intracranial cysts which, most of the time are incidental and disappear with time. Ultrasound of cranium is an easily performed rapid non radiation modality preferred in neonate and infants. The differential diagnosis of intracranial cystic lesions detectable by head ultrasonogram (USG) includes a broad spectrum of conditions. 1.Normal variants, 2.Supratentorial cysts and 3.Infratentorial cysts.

Ultrasonogram can provide important information about the anatomic location, size and shape of the lesions as well as their mass effect on adjacent structures.

1.Normal variants

Normal variants are incidental occurrences and are usually asymptomatic. Imaging features of normal variants simulate a true pathology. Certain intracranial cyst like lesions are normal variants which need no treatment.

Coarctation of the lateral ventricle

This is otherwise called Connatal cyst or frontal horn cyst. On cranial USG (Fig.1a), it appears as thin walled cysts in the superior and lateral aspect of frontal horns of lateral ventricle and anterior to the foramen of Monro, in contrast to the normal frontal horn as in Fig.1b. They are usually multiple cysts noted as string of pearls appearance. The lesions may also be considered as septations related to lateral ventricles adjacent to frontal horns. The lateral ventricle, germinal matrix region and adjacent parenchyma near the lesion all appear normal. The cysts resolve in follow up study.

* Assistant Professor in Radiodiagnosis
** Associate Professor in Radiodiagnosis,
Department of Pediatric Radiology,
Institute of Child Health and Hospital for Children,
Chennai
e mail: raveendran.jayabalan@gmail.com

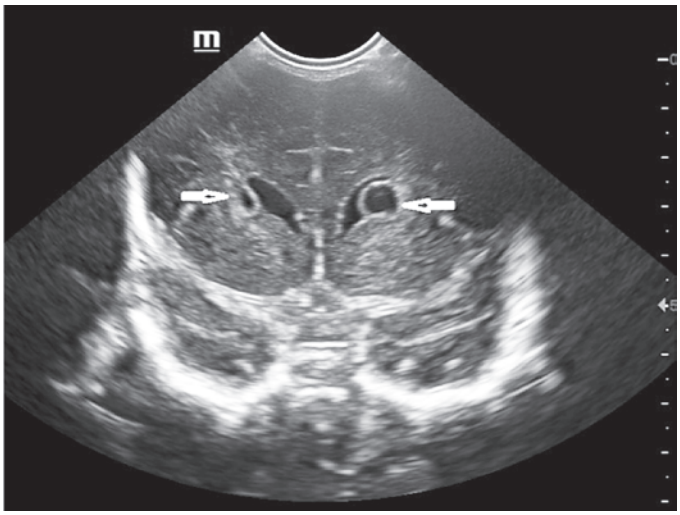


Fig.1a. Coronal view cranial sonography shows thin walled cysts (arrows) in the superior and lateral aspect of frontal horns of lateral ventricle

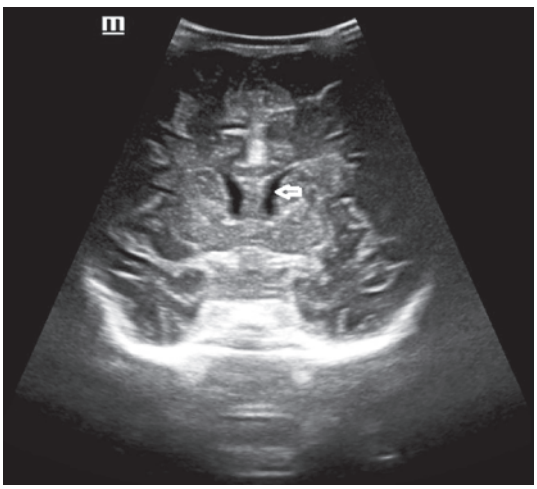


Fig.1b. Coronal view at the level of frontal horn (arrow) for comparison.

Cavum septum pellucidum/Vergae/Veli interpositum

These are normal variant CSF space between the leaflets of the septum pellucidum. It is common in premature infants. It is considered a normal variant due to its frequent appearance and because a specific clinical syndrome has not yet been identified with its occurrence.

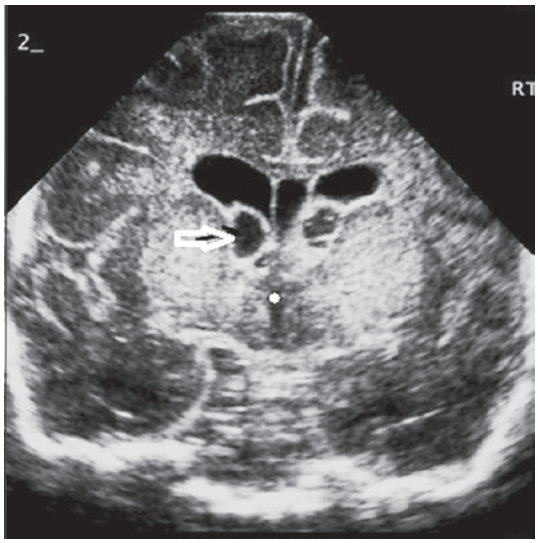


Fig.2. Coronal view cranial sonography shows thin walled cysts (arrows) in the caudate region in periventricular region around the frontal horns of lateral ventricle

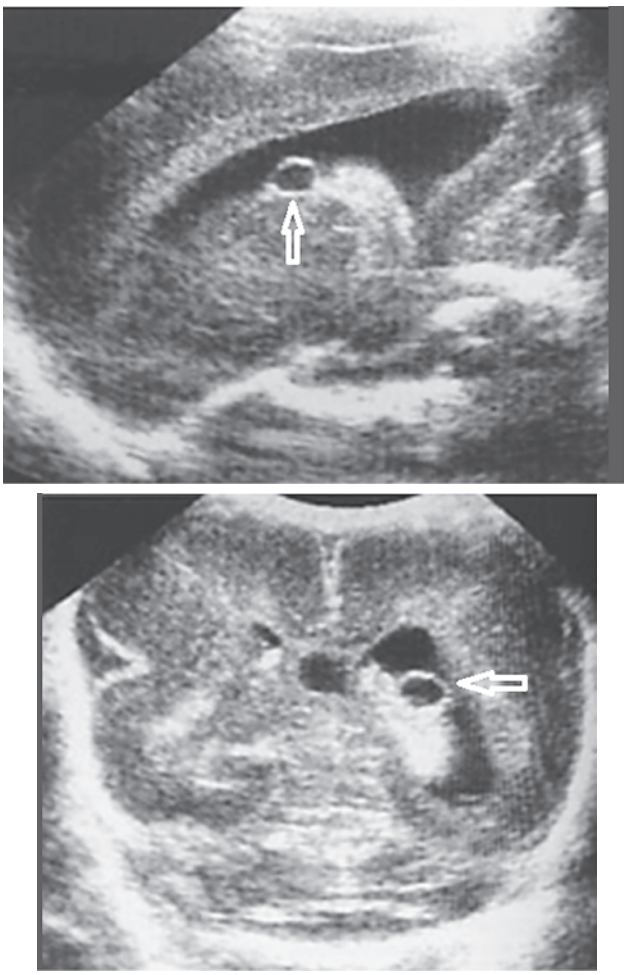


Fig.3. Para sagittal and Coronal view cranial sonography shows thin walled cyst (arrow) in the left choroid plexus of lateral ventricle

The significance of absent cavum septum pellucidum in antenatal imaging is its association with significant CNS anomalies.

2.Supratentorial cysts

Subependymal cysts

These cysts are located in subependymal region around the Caudothalamic notch.These are congenital cysts and represent previous germinal matrix hemorrhage. This reflects germinolysis associated with CMV infection. On cranial USG, they appear as anechoic thin walled cysts (Fig.2) adjacent to the ventricular system in the caudate nucleus or caudothalamic notch.

Choroid plexus cysts

These are cysts located within the body of choroid plexus (Fig.3). They are benign cysts and represent weak marker of aneuploidy. It is more significant when the cysts are large or seen bilaterally. It has no clinical significance if detected after birth. It needs follow up to assess regression of the cyst. On antenatal USG, especially during 2nd trimester, they appear as anechoeic cysts at the level of atria involving the lateral ventricles.

Cystic periventricular leukomalacia

This refers to white matter necrosis seen in preterm infants. On cranial USG, they appear as (Fig.4a) anechoeic lesions dorsal and lateral to external angles of lateral ventricles which are developing cysts, compared to normal appearing choroid plexus at the level of lateral ventricles (Fig.4b)

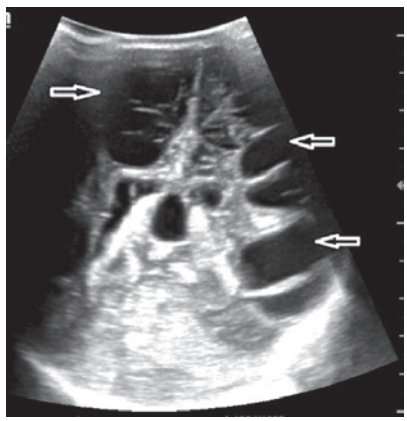


Fig.4a. Coronal view cranial sonography shows anechoeic cystic lesions dorsal and lateral to external angles of lateral ventricles. (arrows).

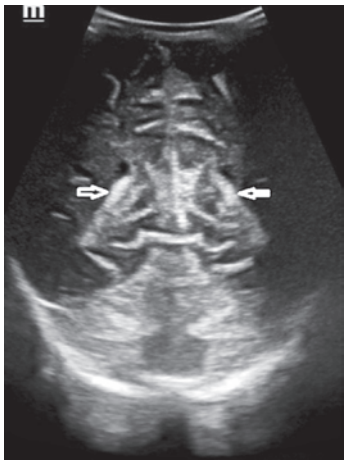


Fig.4b. Coronal view at the level of lateral ventricles showing the choroid plexus (arrows) for comparison

Grading of periventricular leukomalacia

Grade1 - periventricular echoes without any cyst > 7 days.

Grade2 - echoes into small ventricular cysts.

Grade3 - areas of periventricular echoes develop into extensive periventricular cysts in the occipital and fronto parietal region.

Grade4 - periventricular echoes in the deep white matter developing into extensive subcortical cysts.

Porencephalic cyst

These are the areas of cystic encephalomalacia filled with CSF following infection or hemorrhage. On cranial USG. (Fig.5a & b) they appear as one or more intracranial cysts that communicate with the ventricular system and as asymmetrical ventricles with displacement of the midline ventricular echo.

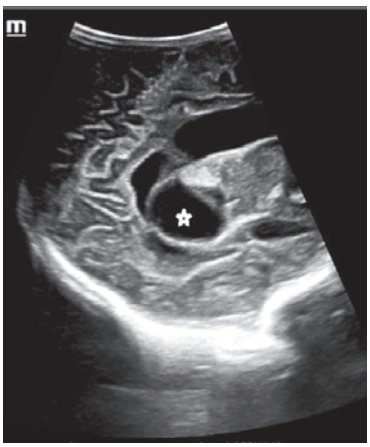


Fig.5a. Para Sagittal view cranial sonography showing porencephalic cyst

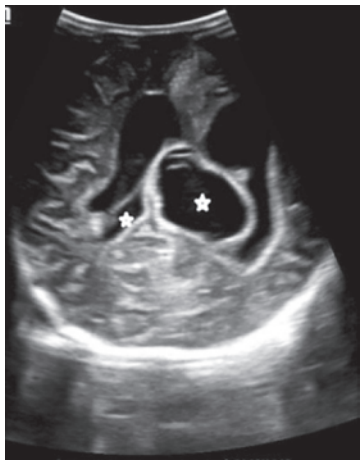


Fig.5b. Coronal view cranial sonography shows intracranial cysts (stars) that communicate with the ventricular system

Arachnoid cyst

Arachnoid cysts account for approximately 1% of all space-occupying lesions. The majority of arachnoid cysts are supratentorial, of which sylvian fissure cysts in the middle cranial fossa are the most common and usually found incidentally.

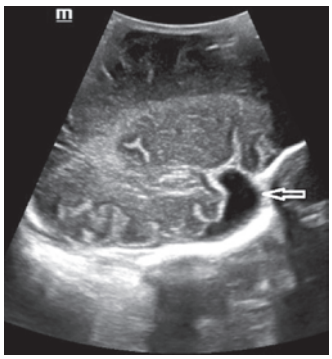


Fig.6a. Para Sagittal view cranial sonography shows the arachnoid cyst (arrow)

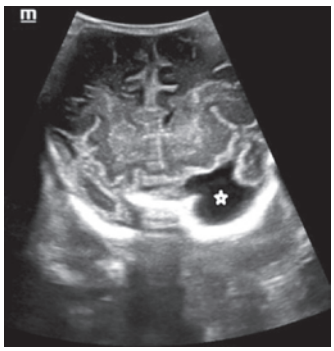


Fig.6b. Coronal view cranial sonography shows arachnoid cyst (star) in middle cranial fossa at left temporal region

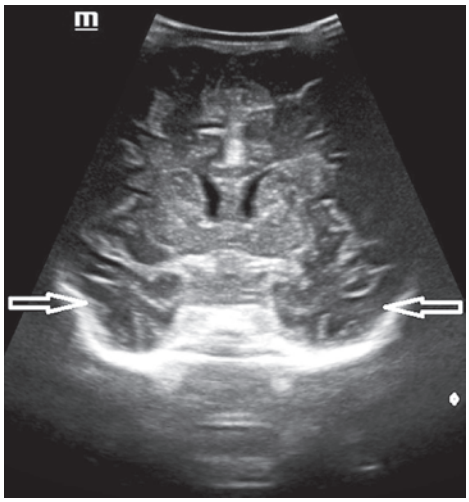


Fig.6c. Coronal view showing the temporal lobes (arrows) for comparison

On cranial USG, arachnoid cysts (Fig.6a & b) are extremely well circumscribed with an imperceptible wall and displaced adjacent structures. They do not show mass effect as they exert a remodelling pattern on adjacent structures and the bone. For comparison, coronal view of the normal temporal lobes is shown in Fig.6c.

The ultrasonogram features of the cystic lesions are correlated with the clinical history. Changes of the appearance of these lesions over time can assist in improving the diagnostic yield. Familiarity with the ultrasonogram features of intracranial cysts is therefore an extremely valuable tool, as it facilitates an accurate diagnosis and treatment when necessary.

Infra tentorial cystic lesions will be discussed under same topic in the subsequent issue.

CLIPPINGS

Engineering a vector-based pan-Leishmania vaccine for humans: proof of principle

Leishmaniasis is a spectrum of diseases transmitted by sand fly vectors that deposit Leishmania spp. parasites in the host skin during blood feeding. Currently, available treatment options are limited, associated with high toxicity and emerging resistance. Even though a vaccine for human leishmaniasis is considered an achievable goal, to date none is available, a probable consequence of lack of pre-clinical to clinical translatability.

Pre-exposure to uninfected sand fly bites or immunization with defined sandfly salivary proteins was shown to reduce infection severity. Still, cross-protection reports are rare and a sandfly saliva-based vaccine will be applicable only to a defined geography, one parasite species and one form of leishmaniasis.

As a proof of principle of a future vector saliva-based pan-Leishmania vaccine, the authors engineered through a reverse vaccinology approach that maximized translation to humans, a fusion protein consisting of immunogenic portions of PdSP15 and LJL143 (sand fly salivary proteins demonstrated as potential vaccine candidates against cutaneous and visceral leishmaniasis, respectively). The in silico analysis was validated ex vivo, through T cell proliferation experiments, proving that the fusion protein (administered as a DNA vaccine) maintained the immunogenicity of both PdSP15 and LJL143. This DNA vaccine was defined as partially protective, in the context of Leishmania major transmission by Lutzomyia. longipalpis sand flies.

Importantly, a high IFNγ response alone was not enough to confer protection, that mainly correlated with low T cell mediated Leishmania-specific IL-4 and IL-10 responses, and consequently with high pro/anti-inflammatory cytokine ratios. Overall their immunogenicity data suggests that to design a potentially safe vector-based pan-Leishmania vaccine, without geographic restrictions and against all forms of leishmaniasis is an achievable goal.

Cecílio P, Oristian J, Meneses C, Serafim TD, Valenzuela JG, da Silva AC, et al. Engineering a vector-based pan-Leishmania vaccine for humans: proof of principle. Sci Rep 10, 18653 (2020). https://doi.org/10.1038/s41598-020-75410-0.

CASE REPORT

A RARE COMPLICATION OF DISTAL RENAL TUBULAR ACIDOSIS

** Yasmeen MS*
*** Avinash Vattam*
**** Mehul A Shah*

Abstract: *We report a 9 year old girl with distal renal tubular acidosis presenting with acute hypokalemia following withdrawal of potassium citrate supplementation. During the course of severe hypokalemia, she developed acute rhabdomyolysis and hyperkalemia, a rare complication. To the best of our knowledge, this is the first report of hypokalemic rhabdomyolysis in a child with distal renal tubular acidosis from India.*

Keywords: *Distal renal tubular acidosis, Hypokalemia, Rhabdomyolysis, Creatine kinase.*

A 9-year girl was diagnosed at 1 year of age with primary distal renal tubular acidosis (dRTA) and nephrocalcinosis. She was adequately treated with sodium bicarbonate and potassium citrate. Due to absence of polyuria, polydipsia and serum potassium of more than 4.4mEq/L consistently for 6 years, low dose potassium citrate treatment was discontinued and she was maintained on oral sodium bicarbonate alone. A month later she presented with severe generalized weakness and leg pain of 3 days duration. History of fever, vomiting, diarrhea, strenuous exercise or missing bicarbonate therapy was denied by parents.

On admission, her weight was 27.6 kg, height 128 cms (25th centile) and systolic BP 100 mmHg. There was generalized hypotonia with power of grade 2-3 in all 4 extremities. Higher cranial functions were intact. Initial tests showed urine pH 8.0, venous pH 7.24dL, serum sodium 143 mEq/L, potassium 1.7 mEq/L, chloride 107 mEq/L, bicarbonate 14 mEq/L, calcium 8.6 mg/dL, phosphorus 4.5 mg/dL, magnesium 1.8 mg/dL, serum albumin 3.7 gram/dL, Vitamin D3 10 ng/ml, blood urea 30 mg/dl and serum creatinine 0.66 mg/dl. She was managed with intravenous and oral potassium supplementation. Her serum potassium increased to 2.7 mEq/L next day. Subsequently, the girl complained of severe leg pains. There was no muscle tenderness. There was acute elevation of serum potassium to 5.8 mEq/L with normal serum creatinine value of 0.6 mg/dL.

Suspecting rhabdomyolysis, serum creatine kinase (CK) was done and found to be elevated at 42,420 U/L (reference 32-180 U/L). Her SGPT was 492 U/L and SGOT 527 IU/L. Her urine colour was clear and heme was negative. She was hydrated with alkalinization and continuation of potassium supplementation was discontinued. The muscle pain reduced on day 4 with simultaneous reduction in serum CK. She was restarted on oral sodium bicarbonate supplementation and she was discharged on day 7 with serum potassium 3.6mEq/L, serum creatinine 0.5mg/dl and serum CK13,950 U/L. Serum CK done a month later was 181 IU/L and potassium citrate was reinitiated.

Discussion

Distal RTA results from defective secretion of H+ in distal nephron and could be inherited or secondary to obstructive uropathy, autoimmune disorders and amphotericin B.[1,2] The major complications of dRTA include growth restriction, hypokalemic paresis and chronic kidney disease.[3] Rhabdomyolysis is a rare complication with severe hypokalemia.[4-6] Congenital renal tubular disorders (Bartter syndrome, dRTA) can cause rhabdomyolysis.[4-5] Often hypokalemia is missed as a cause of rhabdomyolysis leading to rapid development of hyperkalemia. Other causes of rhabdomyolysis include infections, trauma, drugs and myopathies. Von Vigier, et al reported hypokalemic rhabdomyolysis in 14 children with renal tubular disorders (7 with dRTA and 7 with Bartter / Gitelman syndrome) with precipitating factors like respiratory infection, vomiting, or discontinuation of medicine.[5] While potassium wasting can be partially reversed by alkali therapy in dRTA, most patients would

* Consultant Pediatric Nephrologist
** Consultant Pediatrician, Little Stars Children's Hospital
*** Consultant Pediatric Nephrologist, Apollo Health City and Little Stars Children's Hospital, Hyderabad.
email: mehulashah@hotmail.com

need lifelong potassium supplementation.[7] Discontinuation of potassium citrate was the precipitating factor in our patient.

Rhabdomyolysis presents with severe muscular pain, weakness and myoglobinuria. Increased creatine phosphokinase and myoglobinuria are the major laboratory findings. Myoglobinuria may be absent in 25-50% of patients with rhabdomyolysis as myoglobin has shorter half-life of 2-3 hours and rapidly metabolized to bilirubin. Urine dipstick will be positive for heme, only when myoglobin concentration in urine is more than 0.5 to 1 mg/dl.[8] In contrast, CK has a half-life of ~36 hours and declines at a constant rate of 40-50% of the previous day value.

Hypokalemic rhabdomyolysis occurs when serum K^+ is less than 2 mEq/L. During normal muscle activity, there is movement of intracellular potassium into interstitial space of skeletal muscle causing rapid vasodilatation allowing increased blood flow to the muscle. In hypokalemia, potassium accumulation in the interstitial space is impaired thus decreasing the blood flow leading to muscle ischemia and rhabdomyolysis.[6] Low potassium also interferes with the synthesis and storage of glycogen, which is an important source of energy in muscle.

The management of hypokalemic rhabdomyolysis is early detection and adequate hydration with alkalinization to prevent precipitation of myoglobin in renal tubules. Therapy with isotonic saline to maintain urine output of 2-3 ml/kg/hour along with addition of sodium bicarbonate (5-10 meq/500ml fluid) to target urine pH of 6.5 is suggested. Bicarbonate should be discontinued or avoided if there is hypocalcemia (which can aggravate further), serum bicarbonate is > 30 meq/L, or urine pH is >6.5 due to the risk of calcium phosphate deposition in alkaline urine.[9] Often, isotonic saline infusion alone is effective in maintaining diuresis. Diuretics are usually not needed unless there is fluid overload or urine volume is less than 2 ml/kg/hour over 6 hour period. Fluid therapy is usually continued until serum CK is less than 5000 IU/L. In addition, potassium supplementation will be required in alkalotic tubulopathies associated hypokalemic rhabdomyolysis.[5] Rhabdomyolysis can lead to AKI due to tubular injury and obstruction.[6] Von Vogier et al. reported AKI in 7.1% patients with inherited renal tubular disorders and hypokalemic rhabdomyolysis.[5] AKI did not occur in our patient due to early detection and prompt treatment of rhabdomyolysis.

In conclusion, severe rhabdomyolysis following severe hypokalemia in dRTA is not uncommon. Prompt recognition of this entity and appropriate management can prevent AKI, hyperkalemia and hypocalcemia associated with rhabdomyolysis. Continuation of potassium supplementation in these children and prompt treatment of acute gastrointestinal losses will reduce risk of hypokalemia and its complications.

References

1. Palazzo V, Provenzano A, Becherucci F, Sansavini G, MazzinghiB, Orlandini V, et al. The genetic and clinical spectrum of a large cohort of patients with distal renal tubular acidosis. Kidney Int 2017; 91:1243-1255.
2. Lopez-Garcia SC, Emma F, Walsh SB, Fila M, Hooman N, Zaniew M, et al. Treatment and long-term outcome in primary distal renal tubular acidosis. Nephrol Dial Transplant 2019; 34:981-991.
3. Soriano JR. Renal tubular acidosis: the clinical entity. J Am Soc Nephrol 2002; 13:2178-2184.
4. Kim CJ, Woo YJ, Ma JS, Hwang TJ, Kang HC, Kim PK, et al. Hypokalemic paralysis and rhabdomyolysis in distal renal tubular acidosis. Pediatr Int 2005; 47:211-213.
5. Von Vigier RO, Ortisi MT, La Manna A, Bianchetti MG, Bettinelli A. Hypokalemic rhabdomyolysis in congenital tubular disorders: a case series and a systematic review. Pediatr Nephrol 2010; 25:861-866.
6. Al-Ismaili Z, Piccioni M, Zappitelli M. Rhabdomyolysis: pathogenesis of renal injury and management. Pediatr Nephrol 2011; 26:1781-1788.
7. Aronson PS, Giebisch G. Effects of pH on potassium: New explanations for old observations. J Am Soc Nephrol 2011; 22(11):1981-1989.
8. Giannoglou GD, Chatziziis YS, Misirli G. The syndrome of rhabdomyolysis: pathophysiology and diagnosis. Eur J Intern Med 2007; 18(2):90-100.
9. Huerta-Alardín AL, Varon J, Marik PE. Bench-to-bedside review: Rhabdomyolysis - an overview for clinicians. Crit Care 2005; 9(2):158-169.

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