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Editorial

Translating Research into Clinical Practice Skills

Medical Science strives not only to cure diseases but also to prevent illnesses and promoting health. Being healthy increases our productivity and in turn leads to a prosperous nation. Easier said than done because promoting health requires development of skill in utilizing evidence-based clinical practices. This necessitates basic as well as translational and social research. A clinically applicable treatment entails rigorous experimentation before its benefit to the mankind. It has been debated and suggested that the development of an integrated learning healthcare system will have immense impact on the application of clinically relevant medical knowledge for the benefit of present and future patients (1). Systems medicine which has recently been proposed as a future and primary care centred strategy for healthcare worldwide has been discussed in detail and deliberated but likely to remain incomplete as a foundation for clinical understanding and practice (2).

Adolescents are our wealth for the future. Their proper grooming is crucial and important in different stages of life cycle so that they can be instrumental in bringing societal changes towards the positive development of the mankind. It is important because, firstly, they are naïve and open to new information which can be utilized as an agent of change; secondly, they are seeking independence, which if guided suitably through innovative pedagogical principles, will give them desired freedom and at the same time will make them a conscientious and responsible citizen. If anything that affects them most, it is the technological advances which have a direct bearing on adolescents' behaviour as they find it difficult to imbibe and apply it in their day-to-day practice. Harnessing the technology appropriately for adolescents belonging to Generation Z will reap rich dividends in the long run. But it is applicable only well when the teenagers are healthy and free from any physical, mental and social encumbrances and disorders.

National Academy of Medical Sciences (India), as per its mandate, fosters and utilizes academic excellence as its resource to meet medical and social goals. This commitment is met by bringing scientists from varied streams onto a common platform of Academy, from where they can promote need-based research more cohesively for the public health applications. Fellows and Members of the Academy bring rich resources of their longstanding work experience and research. Wisdom shared by these eminent people through Orations and Awards will be remembered as a legacy left behind to the younger colleagues. The present issue of the Annals is an effort in this direction.

The opening article highlights the beneficial effects of intake of vegetables in food and importance of fortified food items together with iron supplementation. Dr. Ramachandran in her study on anemia among teenage girls based on the analyses of data from national surveys conducted over last 2 decades found very small improvement in hemoglobin levels as a time trend. Despite national nutritional programs, this trivial improvement may be explained by poor indicators of living standards and overall

status of the development of the society. She strongly deliberated that Government of India initiative of Double Fortified Salt (DFS) along with increased intake of green vegetables may be economical in reducing prevalence of anemia among adolescent girls and thereby help in improving the health of this important potential workforce of the nation. Even it does not require investment into any costly technology. What it needs is the change in behaviour through well planned educational interventions. The article make us ponder for creating skills in the areas of designing and implementing programs based on Information, Education and Communication (IEC).

Fetal anemia can be lethal unless treated by fetal transfusion. In his Oration in the memory of Dr. JG Jolly delivered during Annual Conference of the National Academy of Medical Sciences (India), NAMSCON 2017 at Amritsar, Brig. (Dr.) Arora presented a detailed account of his work based on managing Rh-alloimmunized pregnancies over a period of 11 years. While reviewing epidemiology, pathophysiology, diagnosis and invasive testing, the Orator went into the details of the procedures for fetal transfusion techniques, thereby saving many infants. The highlight of his study focused on the role of middle cerebral artery-peak systolic velocity (MCA-PSV) using Doppler ultrasound, revealed good correlation with severity of anemia. The favourable neonatal outcome in this study also corroborates with a similar study published elsewhere (3).

Another Oration in the memory of Col. Sangham Lal delivered by Dr. Anil Jain at NAMSCON 2017 at Amritsar reinforces, yet again, the value of Evidence-based Patient Care and also made us ponder- does one research suffice to have evidence towards scientific clinical practice? This article may be able to answer the question to some extent. Dr. Jain, taking an example of tuberculosis of spine, endeavoured to explore questions, accumulating evidences through his continued research in a focused area to find answers for normalising spine and preventing morbidity among his patients. His quest for truth reminisces of a similar study exploring value of medicine-based patient care (4).

For the information of readers, Academy Oration was started in 1965 with a view to inviting one of the senior fellows to share outstanding work done in specific area. Dr. Arvind Bagga, in his Academy Oration delivered at NAMSCON 2017, emphasized the continuing dilemma of children with nephrotic syndrome, a chronic glomerular condition, still with unclear pathogenesis and empirical therapies despite two decades of extensive research. He has shared the recent updates in this review comparing and contrasting various national and international guidelines by different working groups on kidney diseases among children.

Women and children are still a vulnerable group requiring support of the society and target-based provision of healthcare services. Their health in turn builds the healthy society by bridging the gap in the intergenerational cycle. Infant mortality rates in most of the states in India is still very high despite improvement in technologies. Physician still lacks clues to predict preterm birth with certainty and neither have sufficient methods to minimize pregnancy loss which are attributed to multiple factors namely genetic conditions, intrauterine infections or maternal disorders. However, scientific research has vastly improved our understanding of pregnancy and maternal-fetal interactions. In this issue of the Annals, Dr. Kar through his research on Hepatitis E Virus (HEV) reviewed the epidemiology, genotypes and factors responsible for adverse outcome for both mothers and fetus suffering from HEV. He concludes that high viral load and immunological changes together result in poor fetal and maternal

outcomes among pregnant women with HEV. He has also highlighted that pregnancy outcome also depends on prenatal care and maternal nutrition citing studies from developed nations. These studies further through light on the protective effects of maternal nutrition on acute viral hepatitis during pregnancy.

Most articles in this issue primarily highlight the importance of research for the care towards women and children. A healthy teenage will be transformed into an accomplished adult contributing towards a productive society. From academic point of view, skills can be developed or polished in the cognitive (knowledge), psychomotor (procedure) and attitudinal (emotion) areas. However, in the area of health, research skills are going to have tremendous impact as to how we can deliver healthcare. National Academy of Medical Sciences (India) endeavours to build that skill too with the help of a critical mass of Fellows and Members.

From this issue of Annals, we are adding some new features like Letter to the Editor to bridge the gap in communication between the journal and its readers. We welcome correspondence on articles published in the previous two issues as well as invite comments unrelated to articles emphasizing new information or findings not exceeding 400 words. In this issue of Annals, we share a letter highlighting importance of observation in clinical practice through clinical images.

Editorial Board sincerely hope that Annals will live up to the expectations of the readers and the society which it serves. The official journal is also available free online and does not charge for publication. Scientific contributions from the fellows, members, readers and healthcare professionals on various topics from all streams of biomedical sciences are always welcome but will be subjected to usual review process to enhance the quality and authencity.

Kuldeep Singh
Sanjeev Misra

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Time Trends in Prevalence of Anaemia in Adolescent Girls

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ABSTRACT

Introduction: Anaemia in adolescent girls has been recognised as a major public health problem. The Mid-day meal programme guidelines envisage inclusion of 75 g/day of vegetables and use of iron fortified iodised salt for hot cooked meal. The National Iron Plus Initiative envisages weekly iron-folic acid (IFA) supplementation for adolescent girls; however, coverage and compliance have been reported to be low. Data from national surveys carried out in the last two decades were analysed to assess changes, if any, in Hb levels and prevalence of anaemia in adolescent girls.

Material and Methods: Raw data from National Family Health Surveys (NFHS) -2, -3, and -4, District Level Household Surveys (DLHS) 2 and 4, and Annual Health Survey-related to Clinical, Anthropometric and Biochemical Components (AHS-CAB) were analysed to assess mean Hb, prevalence of anaemia and frequency distribution of Hb in adolescent girls. Comparison in these parameters was made between non-pregnant girls 10-14 years and 15-19 years of age in DLHS-2, -4 and AHS-CAB; in the 15-19 year age group comparisons were made between pregnant and non-pregnant girls in NFHS series and DLHS AHS series.

Results: There were no clear and consistent changes in mean Hb, prevalence of anaemia and frequency distribution of Hb in pregnant and non-pregnant adolescent girls between NFHS-2, -3 and -4 either at national or at State level. However, there was a 0.7 and 1.3 g/dL increase in mean Hb levels in non-pregnant girls (10-19 yrs) between DLHS-2 and AHS-CAB and DLHS-4 States, respectively. The increase in mean Hb of pregnant girls (15-19 yrs) was 1.1 g/dL and 1.4g/dL in AHS-CAB and DLHS 4 States, respectively. There was significant reduction in prevalence of anaemia in both pregnant and non-pregnant girls between DLHS 2 and DLHS 4 and AHS-CAB at the aggregate level for each survey and in all States except Uttarakhand.

Conclusion: There has been some improvement in Hb levels in adolescent girls in the last two decades. Improving dietary intake of vegetables and promoting use of iron fortified iodised salt in all households in the country have to be taken up so that iron intake across all age groups improves. This when combined with daily IFA supplementation for three months in a year in adolescent girls, might lead to sustained improvement in Hb.

Keywords: Iron and Folic Acid supplementation, dietary intake of iron, dietary diversification, double fortified salt.

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Introduction

India had recognized that anaemia in adolescent girls was a major public health problem with adverse consequences to the girl and, if she conceives, her child. Research studies in India had documented that infants born to anaemic mothers had poor iron stores. Prevalence of anaemia in children is high because of poor dietary intake of iron and folate, and poor bio-availability of iron from Indian dietaries. Efforts are being made to increase iron intake through inclusion of vegetables and iron fortified iodised salt in the hot cooked meal in mid-day meal programme. Increased requirement of haematopoietic nutrients during adolescent growth and onset of menstruation aggravate anaemia in adolescent girls. Early marriage and pregnancy not only perpetuate anaemia in adolescent girls but result in adverse health consequences for the mother-child dyad. In view of the high prevalence of anaemia in adolescent girls (1-9), studies were taken up to assess the feasibility, acceptance, compliance, continuation rates and impact of daily, bi-weekly and weekly supplementation of iron (100 mg) and folic acid (500 μg) to adolescent girls (10-18). These research studies showed that school-based once-a-week supervised iron-folic acid (IFA) supplementation was feasible; supplementation over one year resulted in improvement of iron stores and Hb by 0.5 g/dL. Based on the encouraging results from research studies, Ministry of Health and Family Welfare, Govt of India, initiated the supervised Weekly IFA Supplementation (WIFS) Programme to reduce the prevalence and severity of anaemia in adolescent school girls (19). The programme has been implemented across the country both in rural and urban areas. Data from large scale national surveys were analysed to assess changes, if any, in Hb status of adolescent girls in the last two decades.

Material and methods

In the last two decades Hb estimation in pregnant and non-pregnant women and adolescent girls had been undertaken by National Family Health Surveys (NFHS)-2 (1998-99) (1), -3 (2005-06) (2), and -4 (2015) (3), District Level Household Surveys (DLHS)-2 (2002-04) (6), -4 (2013-14) (7) and Clinical and Anthropometric and Biochemical (CAB) Component of the Annual Health Survey (AHS 2014-15) (AHS-CAB) (8). Prevalence of anaemia was computed using the cut-off Hb of 12 g/dL in non-pregnant and 11 g/dL in pregnant girls.

NFHS undertook the survey in pregnant and non-pregnant girls between 15 and 19 years of age and used Haemacue method for estimation of Hb. Raw data from NFHS 2, 3 and 4 were obtained from Demographic and Health Survey Programme ICF International. Only those adolescent girls who had Hb levels between 2.5 to 18.0 g/dL were included for the analysis. Actual Hb values without any weightage and without any correction for altitude or smoking were used for analysis. Data from NFHS 2, 3 and 4 were analysed for mean Hb levels, prevalence of anaemia and frequency distribution of Hb at national and State level for both pregnant and non-pregnant adolescent girls.

Raw data of DLHS-2 and -4 were obtained from International Institute for Population Sciences (IIPS), Mumbai; raw data of AHS-CAB was obtained from Ministry of Health and Family Welfare Govt of India. DLHS-2 covered all the States and UTs; AHS-CAB covered 9 poorly performing States (AHS-CAB States), while DLHS 4 covered 21 States and UTs (DLHS 4 States). DLHS 2, 4 and AHS-CAB surveyed the girls between 10 and 19 years. Hb estimation in DLHS 2, 4 and AHS-CAB was carried out using cyanmethaemoglobin method. Only those adolescent girls who had Hb levels between 2.5 and 18.0 g/dL were included for the analysis. Actual Hb values without any weightage were used for analysis. The mean Hb, prevalence of anaemia and frequency distribution of Hb in adolescent pregnant and non-pregnant girls in the 15-19 year age group

were computed in DLHS 2 and 4 and AHS-CAB. The mean Hb, prevalence of anaemia and frequency distribution of Hb in non-pregnant girls in 10-14 year and 15-19 year and pregnant girls in 15-19 year age groups in DLHS 2 were compared with the prevalence of anaemia and frequency distribution of Hb in the corresponding groups in DLHS-4 and AHS-CAB.

Table 1: Number of adolescent girls

Age	Survey	Total No. surveyed	No. of blood samples collected	No. with valid Hb
10-14 year (Non-pregnant)	DLHS 2 (AHS States)	95245	57275	51070
	AHS States	100794	64967	64960
	DLHS 2 (DLHS 4 States)	62386	36506	33200
	DLHS 4 States	51380	47610	43461
15-19 year (Non-pregnant)	DLHS 2 (AHS States)	72719	41948	37540
	AHS States	102797	61463	61461
	DLHS 2 (DLHS 4 States)	56920	30913	28380
	DLHS 4 States	53030	50571	46459
	NFHS 2	3089	2686	2682
	NFHS 3	2326	2144	2144
	NFHS 4	119655	116615	116564
15-19 year (Pregnant)	DLHS 2 (AHS States)	3850	2682	2295
	AHS States	1332	1025	1024
	DLHS 2 (DLHS 4 States)	2145	1589	1452
	DLHS 4 States	1210	1172	1056
	NFHS 2	444	392	392
	NFHS 3	319	292	292
	NFHS 4	3652	3573	3571

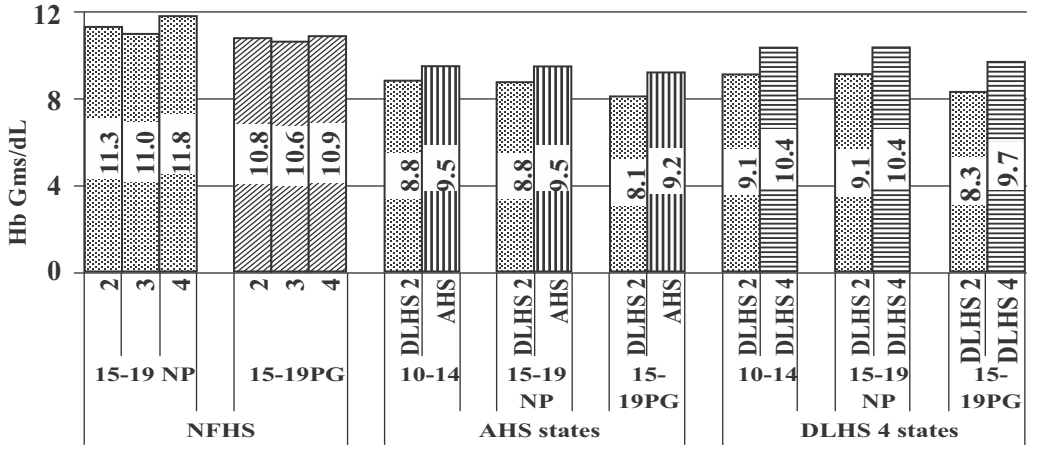
Results

The number of adolescent girls (pregnant and non-pregnant) surveyed, blood sample collected and valid Hb results available in different surveys are shown in Table 1.

Mean Hb levels in pregnant girls were lower as compared to the non-pregnant girls in NFHS 2, 3 and 4. Mean Hb level in non-pregnant and pregnant girls were lower in NFHS 3 as compared to NFHS 2. Mean Hb in non-pregnant girls in NFHS 4 was higher as compared to the mean Hb level in NFHS 2 and 3. However, there were no differences in the mean Hb levels in pregnant adolescent girls between the three surveys (Fig. 1).

The mean Hb in pregnant girls was lower than the mean Hb in the non-pregnant girls in all the surveys. The mean Hb in the AHS States both in non-pregnant and pregnant girls were lower as compared to the corresponding groups in DLHS 4 States. There was substantial improvement in the mean Hb both in non-pregnant and pregnant girls over time in both AHS and DLHS 4 States. The improvement in mean Hb level was lower in AHS States: 0.7 g/dL in non-pregnant and

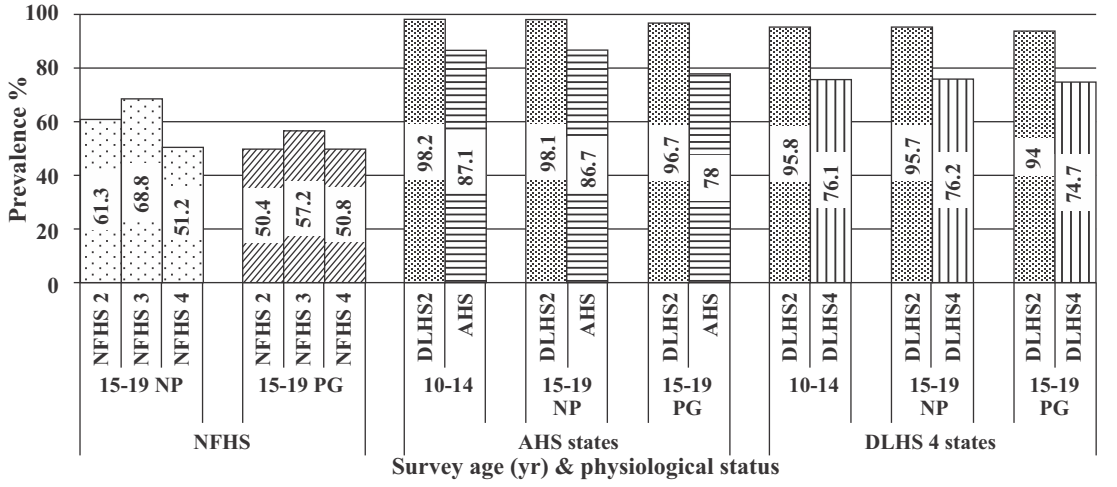
1.1g/dL in the pregnant girls. In DLHS 4 States the improvement was 1.3 g/dL in non-pregnant and 1.4 g/dL in the pregnant women (Fig. 1).



95% CI	NFHS 2	NFHS 3	NFHS 4	DLHS 2	AHS CAB	DLHS 2	DLHS 4
15-19 yr girls (NP)	11.23-11.37	10.97-11.11	11.79-11.81	8.78-8.82	9.48-9.52	9.08-9.12	10..38-10.42
15-19 yr girls (PG)	10.65-10.98	10.41-10.81	10.85-10.95	8.08-8.20	9.06-9.34	8.25-8.43	9.57-9.83
10-14 yr girls (NP)	NA	NA	NA	8.81-8.83	9.48-9.52	9.10-9.14	10.38-10.42

Fig.1 : Mean Hb in adolescent girls

Prevalence of anaemia in pregnant and non-pregnant girls in NFHS 2, 3 and 4 are given in Fig 2. Prevalence of anaemia in pregnant and non-pregnant girls was similar in NFHS 4. Prevalence of anaemia was higher in both pregnant and non-pregnant girls in NFHS 3 as compared to NFHS 2; prevalence of anaemia in pregnant and non-pregnant girls was lower in NFHS 4 as compared to both NFHS 2 and 3 (Fig. 2).



95% CI	NFHS 2	NFHS 3	NFHS 4	DLHS 2	AHS CAB	DLHS 2	DLHS 4
15-19 yr girls (NP)	59.45-63.14	66.84-70.76	50.87-51.45	98.00-98.27	86.45-86.98	95.51-95.98	75.79-76.56
15-19 yr girls (PG)	45.44-55.32	51.51-62.87	48.47-53.07	95.96-97.42	75.49-80.56	92.79-95.23	72.09-77.34
10-14 yr girls (NP)	NA	NA	NA	98.07-98.30	86.82-87.34	95.58-96.01	75.64-76.56

Fig.2 : Prevalence of anaemia in adolescent girls

Comparison of prevalence of anaemia in non-pregnant girls (10-14 and 15-19 years) and pregnant girls (15-19 years) in DLHS 2 with prevalence of anaemia in DLHS 4 and AHS-CAB in these groups showed that there was substantial reduction in prevalence of anaemia to the right of both NFHS 2 and 3 (Fig. 3). Comparison of frequency distribution of Hb in non-pregnant girls (15-19 years) in DLHS 2 and AHS-CAB and DLHS 4 States showed that there was a clear shift to the right in Hb frequency distribution in the latter surveys (Fig. 3). While

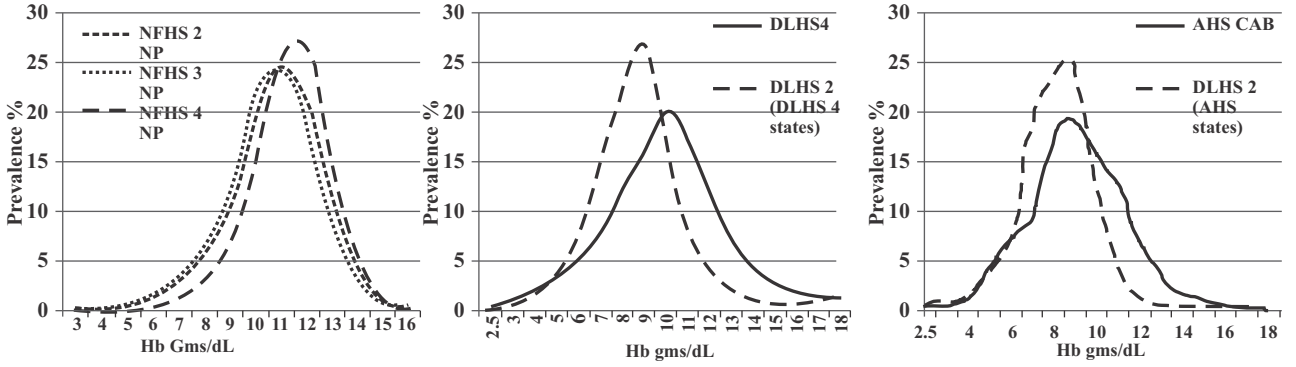


Fig.3: Frequency distribution of Hb in 15-19 yr non-pregnant girls

between DLHS 2 and AHS-CAB and DLHS 4 both in non-pregnant and pregnant girls. The difference in prevalence of anaemia in pregnant and non-pregnant girls in all the surveys was small except in AHS-CAB (Fig. 2.)

Frequency distribution of Hb in different surveys is shown in Fig. 3, 4 and 5. There was shift to the left in the frequency distribution of Hb in 15-19 year non-pregnant girls in NFHS 3 as compared to NFHS 2; in NFHS 4 the shift was to the right of both NFHS 2 and 3 (Fig. 3). Comparison of frequency distribution of Hb in non-pregnant girls (15-19 years) in DLHS 2 and AHS-CAB and DLHS 4 States showed that there was a clear shift to the right in Hb frequency distribution in the latter surveys (Fig. 3). While there was no shift in the frequency distribution of Hb in pregnant adolescent girls in NFHS, a clear shift to the right in frequency

distribution of Hb was seen in pregnant adolescent girls in DLHS-2 and DLHS 4 and AHS- CAB (Fig. 4). The frequency distribution of Hb in non-pregnant girl in the age group 10-19 years in DLHS 4 and AHS-CAB showed clear shift to the right as compared to DLHS 2 (Fig. 5).

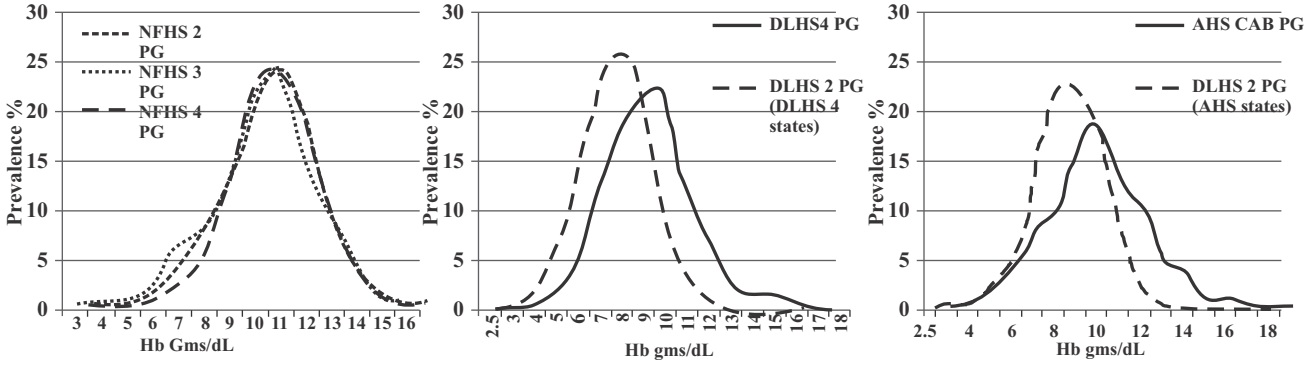


Fig.4 : Frequency distribution of Hb in 15-19 yr pregnant girls

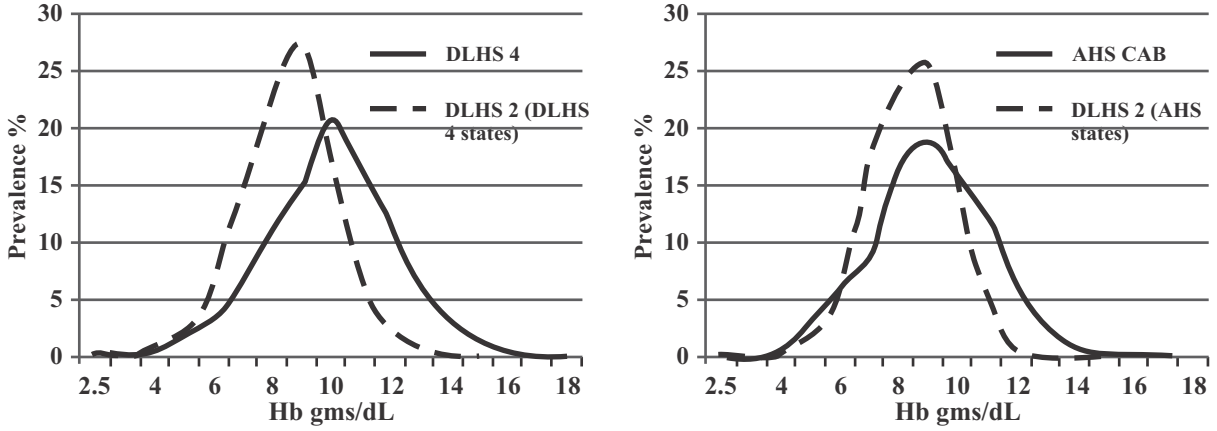


Fig.5 : Frequency distribution of Hb in 10-14 yr girls

There were substantial inter-state differences in the prevalence of anaemia in non-pregnant adolescent girls in NFHS and in DLHS and AHS- CAB States. However, in NFHS series none of the States showed consistent or significant reduction in prevalence of anaemia in non-pregnant adolescent girls. Prevalence of anaemia in adolescent girls was lower in DLHS 4 States as compared to AHS-CAB States at both the time points. In all States except Uttarakhand prevalence of anaemia was lower in AHS-CAB and DLHS 4 as compared to the prevalence in DLHS 2 in these States (Fig. 6).

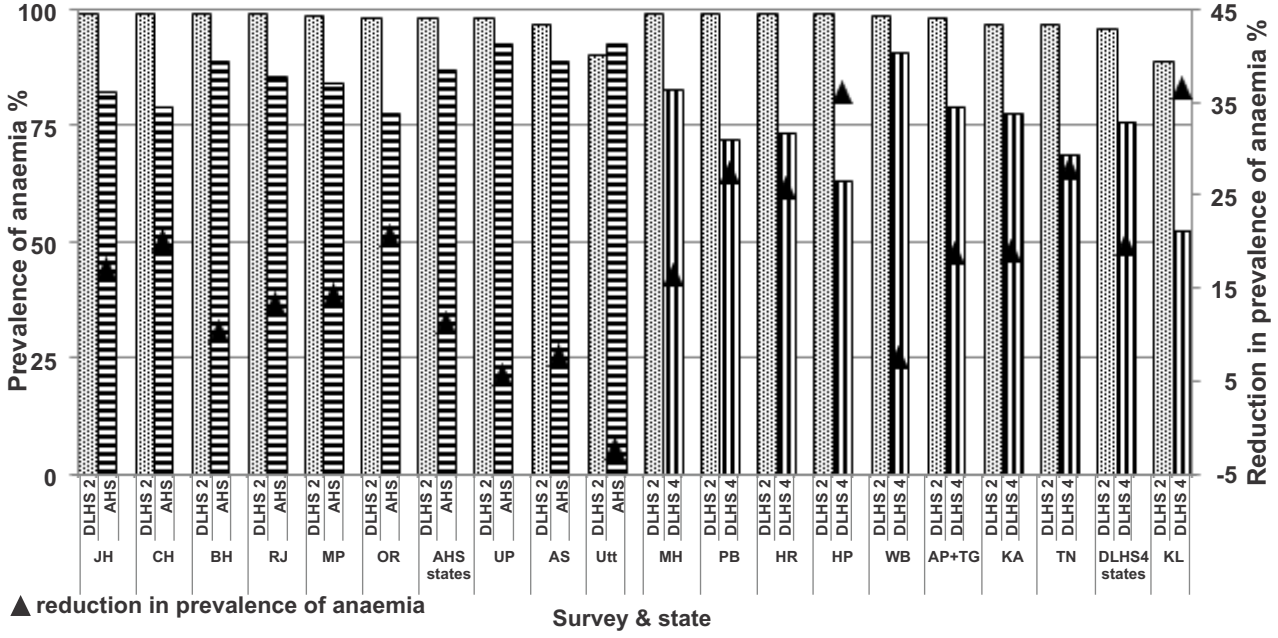


Fig.6 : Inter-state difference in prevalence of anaemia in 10-19 yr non-pregnantt girls

Discussion

In India dietary intake of vegetables rich in iron and folate is low; bioavailability of iron is poor because of the high phytate and fibre contents of the diet. Therefore, right from the childhood, the prevalence of anaemia is high. Adolescent girls require additional iron and other nutrients to meet the needs for adolescent growth spurt and onset of menstruation. These requirements cannot be met from conventional habitual diets and, therefore, there is increase in prevalence and severity of anaemia in adolescent girls. Early marriage and advent of pregnancy in adolescent girls aggravates anaemia further and can result in adverse consequences to the mother-child dyad. IAF supplementation in adolescent girls might reduce the prevalence and severity of anaemia in them and may even help them in becoming non-anaemic before they become pregnant.

Research studies in India have shown that weekly IFA supplementation is feasible and brings about an improvement in mean Hb by 0.5 g/dL/year. With increasing proportion of girls attending the upper primary and secondary schools, they can be reached and provided WIFS supplements readily in schools; out-of-school adolescent girls could be contacted through Integrated Child Development Services (ICDS) programme in anganwadis and given WIFS supplements. In view of this Govt of India has initiated the nationwide WIFS Programme in 2015. However, available meagre reports suggest that coverage under the programme was suboptimal in many States. The suboptimal coverage is partly due to side effects of iron and partly due to the problems in sustaining round-the-year supplementation year after year for a problem which is asymptomatic and where improvement is not perceptible to the person receiving the supplement. It is essential to find out whether there has been any improvement in Hb status of adolescent girls over the last two decades so that appropriate mid-course corrections can be done in the on-going interventions.

Data from NFHS, DLHS and AHS were analysed to assess changes, if any in mean Hb, prevalence of anaemia and frequency distribution of Hb in adolescent girls in the last two decades. Data from the NFHS surveys showed that between NFHS 2 and 3 that there was a small decline in mean Hb (Fig. 1) and 5% increase in prevalence of anaemia (Fig. 2); between NFHS 3 and 4 there was an improvement in mean Hb and 10% reduction in prevalence of anaemia (Fig. 1 and 2). The reason for the higher prevalence of anaemia in NFHS 3 is not clear. The data from the NFHS 2, 3 and 4 showed that improvement in Hb levels and reduction in prevalence anaemia in adolescent girls was small.

NFHS data are widely used by academics and programme managers to assess time trends in access to health and nutrition services and progress in terms of improvement in health and nutritional status. The data from NFHS showing lack of change in Hb status of adolescent girls over the last two decades was interpreted as being due to poor coverage and compliance with the WIFS programme for the adolescent girls. However, it is possible that the reported lack of improvement in Hb could, at least in part, be due to the problems in the method (Haemacue) used for Hb estimation in NFHS 2, 3, and 4. There have been publications indicating that Haemacue does not estimate Hb accurately (20-23).

To explore this possibility, comparison was made with data on mean Hb, prevalence of anaemia and frequency distribution of Hb from DLHS 2 and 4 and AHS-CAB surveys which were undertaken during the same period and used the gold standard cyanmethaemoglobin method for Hb estimation. Hb data from DLHS 4 and AHS-CAB showed that as compared to DLHS 2, there was an increase in mean Hb, reduction in prevalence of anaemia, and shift to the right in frequency distribution of Hb both in non-pregnant (10-14 years and 15-19 years of age) and pregnant (15-19 years) adolescent girls (Fig. 1-5) in both AHS-CAB and DLHS 4 (for

their respective States). It was reassuring to note that there has been some improvement in Hb status of adolescent girls, despite the fact that the national level Weekly IFS supplementation (WIFS) programme was initiated only in 2015 and the reported coverage under the programme was low.

Over the last two decades, there has been substantial improvement in per capita income, reduction in poverty and improvement in household food security and a slow but steady decline in under-nutrition rates; access to healthcare for malaria and hook worm infestation has improved. It is possible that the observed improvement in mean Hb and reduction in prevalence of anaemia both in non-pregnant and pregnant adolescent girls might be part of the overall improvement in nutrition and health status of adolescent girls. It is, however, a matter of concern that prevalence of anaemia across all States of the country continues to be unacceptably high. There is an urgent need to accelerate the pace of improvement in Hb and reduction in prevalence of anaemia in adolescent girls using all available interventions.

The WHO had consistently recommended oral iron supplementation as a public health intervention for improving Hb and iron status and reducing the prevalence of anaemia in adolescent girls. Systematic reviews of the randomized clinical trials with IFS supplementation in adolescent girls (24) have shown that even in situations where prevalence of anaemia was high, IFS supplementation for three months or longer resulted in improvement in mean Hb (of about 0.5 to 1 g/dL). Improvement in Hb and ferritin levels was higher in daily supplementation as compared to biweekly or weekly supplementation, but compliance with daily supplementation was difficult to maintain on long term basis. Taking the operational difficulty in daily supervised administration of IFS tablets to adolescent girls, the earlier WHO guidelines had recommended weekly IFA supplementation (25). However, such intermittent supplementation has to be continued throughout the year, and year after year. Round-the-year supervised weekly IFA supplementation may pose problems in many settings. The current WHO guidelines recommend daily IFA supplementation for 3 months every year in settings where prevalence of anaemia is 40% or higher (26).

In the Indian context it may not be possible to rely solely on continued IFA supplementation to adolescent girls to achieve sustained reduction in prevalence of anaemia. Supplementation programmes are expensive because personnel are required for counselling, distributing supplements and monitoring to improve compliance. It has been well documented that not all anaemic persons become non-anaemic after three-months of daily supplementation; once supplementation is stopped some of those who became non-anaemic may become anaemic (27-30). Taking these into consideration, the WHO advocates food fortification with iron for sustained improvement in iron intake in countries with low iron intake (31). In India, low iron intake and poor bioavailability of iron from Indian diets are the major factors responsible for the high prevalence of anaemia across all age and physiological groups. The National Iron Plus Initiative guidelines recommend increasing vegetable intake and use of iron fortified iodised salt (double fortified DFS) at household level (32). Government of India's guidelines mandate use of DFS and vegetables in the mid-day meal programme for school children and hot cooked meal provided to the 3-5 years old children under ICDS; efforts are being made to fully operationalize these guidelines. Simultaneously, efforts to improve vegetable intake and promote use of DFS in all households in the country have to be taken-up so that iron intake across all age groups improves. This when combined with daily IFA supplementation for three months in a year in adolescent girls, might lead to sustained improvement in Hb. These efforts in reducing prevalence of anaemia in adolescent girls will, in the long run, enable the country to move towards Sustainable Development Goal (SDG) target of 50% reduction in anaemia in women.

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Fetal Blood Transfusion: The Saviour

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ABSTRACT

The purpose of this oration is to discuss the modality of highly specialized Intra vascular fetal blood transfusion and its various sites to perform fetal blood transfusion with the role of middle cerebral artery-peak systolic velocity (MCA-PSV), as measured by Doppler ultrasound, in managing fetal anemia in Rh-alloimmunized pregnancies. Intra-uterine fetal blood transfusion was performed in such anemic fetuses to tide over the crisis of fetal immaturity till considered fit for extra-uterine survival. Rh-alloimmunized pregnancies with or without hydrops reporting to our tertiary care institute from January, 2005 to December, 2015 were screened by Doppler ultrasound to estimate MCA-PSV to detect fetal anemia. During follow-up, if the fetus developed MCA-PSV values more than 1.5 MoM for the gestational age, fetal blood sampling through cordocentesis was performed to confirm fetal anemia. This was followed by intra-uterine fetal blood transfusion to all the anemic fetuses at the same sitting. The neonatal outcome was evaluated by recording gestational age at the time of delivery, duration of gestational time gained, and need for blood transfusion in the neonatal period. A total of 226 Rh-alloimmunized pregnancies were evaluated. Three hundred ninety six intra-uterine fetal blood transfusions were performed. In their neonatal period, 137 neonates received blood transfusion. Intra-uterine fetal death occurred in 11 fetuses out of which 7 were grossly hydropic fetus. Favorable neonatal outcome was recorded in the rest including 42 hydropic fetuses. The clinical outcome of these pregnancies justifies the use of Doppler studies of MCA-PSV in detecting fetal anemia as these were found to correlate well. Intra-uterine fetal blood transfusion in the anemic fetuses is the only hope of prolonging pregnancy salvaging such fetuses.

Keywords: Intra-uterine fetal blood transfusion, Rh-alloimmunization, hemolytic disease of fetus and newborn, fetal anemia, MCA-PSV, cordocentesis, neonatal outcome.

Introduction

The hemolytic disease of the fetus and newborn (HDFN) has now largely supplanted the more clumsy and less euphonic name "erythroblastosis fetalis". The essential underlying pathology is an active hemolysis of the Rh D positive fetal red cells before, at, and after birth. The three conditions hydrops fetalis, icterus gravis neonatorum, and hemolytic anemia of the newborn are recognized to differ only in the degree and are related to one disease process. Dr. Ian Donald (Regius Professor of Midwifery at Glasgow University) quotes that the recognition of Rh factor, its clinical importance and practical applications of this

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DR. J.G. JOLLY ORATION delivered during NAMSCON 2017 at Sri Guru Ram Das Institute of Medical Sciences & Research, Sri Amritsar, Punjab.

knowledge in fetal therapy as one of the great romances of modern research where theory, observation and practice have, in the space of few years, pieced themselves together to form a coherent picture.

HDFN secondary to maternal rhesus (Rh) isoimmunization or alloimmunization was once a major contributor to perinatal morbidity and mortality. The Rh factor, a blood group D antigen is the main cause of hemolytic anemia of the newborn. The outcome is manifested by hemolytic anemia and/or jaundice in the newborn or hydrops (severe edematous swelling) or a stillborn baby. The disease is produced when a mother is of blood group Rh D negative, the father is Rh D positive and the conceived baby inherits the father's Rh D positive blood group. There is feto-maternal hemorrhage during pregnancy. The baby's blood group is incompatible with that of the mother and generates an immunological response against paternally derived red blood cell antigen D foreign to the mother but inherited by the fetus in the form of antibodies from placental cross over. These maternal antibodies response escapes in first pregnancy but in subsequent pregnancies, if the fetus is Rh D positive, the antibodies cross the placenta and destroy the baby's Rh positive red blood cells resulting frequently fetal anemia, hemolytic jaundice of new born, hydrops, stillbirth, etc. This hemolytic disease can be prevented if the Rh D negative mother has not been sensitized (developed antibodies). This can be accomplished by giving an injection of sterilized blood product called Rh immunoglobulin. Today, administration of Rh immunoglobulin has markedly decreased the prevalence of fetal hemolytic disease such that less than three cases occur in every 1000 live births (1). Failure of Rh immuno-prophylaxis still occurs as a result of inadequate use of Rh immune prophylaxis after potential sensitizing events and administration of inadequate dose. The clinician must be alert to detect Rh alloimmunization because it still constitutes significant proportion of perinatal mortality. Rhesus disease will have a favourable outcome in most instances if properly managed. The rarity of this condition warrants consideration of consultation or referral to a maternal-fetal medicine specialist. The introduction of high-resolution real time ultrasound and the development of intra uterine fetal blood transfusion performed intra-vascular has improved the prognosis optimally even in severely alloimmunized women with hydropic fetus. The reduction in perinatal deaths due to HDFN has also occurred as a result of the great advances made in neonatal care and in the management of the affected fetus.

Evaluation for the presence of maternal anti-D antibody should be undertaken at the first prenatal visit by performing Indirect Coombs Test. If there are no antibodies there is no Rh alloimmunization. Once sensitization occurs, Rh immune globulin is no longer effective. First-time sensitized pregnancies are followed with serial maternal titers. Maternal antibody titers cannot be used to screen for fetal anemia in affected pregnancies but only suggest severity of the HDFN. If antibodies are present in critical levels then various degree of fetal affection will occur if fetus is Rh-positive but will escape fetal anemia if the fetus is Rh D negative.

About 5% of the Indian population is Rh D negative. When prophylactic anti-D immunoglobulin was not available, the incidence of Rh D immunization was reported by the Institute of Immunohematology as 4 to 5% among Rh D negative women registered in the outpatient department of a tertiary care hospital at Mumbai (2).

History

In 1941, Levine et al, demonstrated that the maternal Rh antibodies were responsible for causing erythroblastosis fetalis (3). Soon, after this exchange transfusion was shown to improve the prognosis for the affected neonate. A study by Bevis showed that the severity of the fetal

hemolytic disease could be predicted by serial amniocentesis (4). A major therapeutic breakthrough came in 1963 when Sir William Liley successfully performed the first intra-uterine fetal blood transfusion in severely affected fetus by intraperitoneal route using adult blood cells with fluoroscopic control (5). At about the same time several attempts were made to perform intravascular fetal blood transfusion by hysterotomy with very high maternal and fetal risks. Twenty years later, Rodeck et al reported a percutaneous technique for intravascular fetal blood transfusion using fetoscopy (6). Daffos *et al* introduced fetal blood sampling in 1983 under ultrasound guidance that has now become a routine procedure and guided the preferred technique for fetal blood transfusion (7). The development of anti-D immunoglobulin in 1961 fortunately avoids most Rh-negative women with such fetal intervention (8).

Genetics

The Rh blood group antigens are present as distinct trans-membrane proteins on the red blood cell (RBC) surface (9). Fischer and Race first proposed the concept of three genes that encode for the three major Rh antigen groups – D, C/c and E/e in 1946 (10). The *Rh* gene locus on short arm of chromosome one at 1p34 – p36 locus was discovered 45 years later by Cherif and Zahar in 1991 (11). The *Rh* gene consists of two closely linked and homologus genes with 96% similarity with each of 10 exons in length (12). One gene is designated as *RhCcEe* that encodes both Cc and Ee proteins. The second gene *Rh D* encodes the major antigen Rh D. An *Rh D* pseudogene has also been described (13) in which all the exons of the *Rh D* gene are present but the translation of the gene into a messenger RNA does not occur due to presence of stop codon. Since no Rh D protein is synthesized, the individual is serologically Rh D negative. The presence of such scenario in Rh D negative patient has implications in prenatal diagnosis of fetal blood group by polymerase chain reaction (PCR) on small amounts of fetal DNA obtained by amniotic fluid or chorionic villus sampling. Finally the serological Rh D negative means the absence of the major D antigen on the red cell surface either as a result of homozygous absence of *Rh D* gene or partial deletion of exons 7 to 9 or a stop codon exon 5 of the *Rh D* gene (14).

There are as many as 40 other Rh antigens but the Rh D epitope is the most immunogenic of the Rh system, followed by Rhc that is more than 20 folds less potent. The proportion of people who are Rh D negative varies according to race. The highest Rh D negative individuals are found in the Basque population from Spain which is as high as 30%. Dr Ian Donald observed that in China, Rh D negative population is most uncommon and suggested that the older civilization of Chinese has by process of natural selection bred out the undesirable gene. In China and Japan it is rare to find Rh D negative individual as the incidence drops to less than 1%. However, about 15% Caucasian population is Rh D negative compared to Afro-Caribbean black population who are 7 – 8% Rh D negative.

Pathophysiology

Red cell membrane contains numerous surface antigens including those of ABO and Rh groups. Maternal IgG antibodies can be generated against most of these antigens following exposure to an adequate quantity of red cell antigen due to transplacental fetomaternal hemorrhage or after heterologus blood transfusion. It has been demonstrated that around 75% women have fetal red cells in their circulation of variable numbers depending on period of gestation (15). For practical purposes anti-Rh D, anti-c, anti-E and anti-Kell antibodies have potential to cause moderate to severe HDFN (16, 17). Initial exposure to foreign red blood cell antigen leads to a primary immune response in the mother producing IgM antibody after a latent period of few weeks. After the D antigenic stimulus, IgM anti-D antibodies having the molecular weight of 90,000 daltons appears first. These IgM antibodies cannot cross the placenta and the fetus remain unaffected. On

exposure of the mother to similar antigenic red cells during subsequent pregnancy the previously primed memory B cells produce IgG antibody. A considerably less antigenic stimulus is required for this secondary response compared to the primary exposure and much higher antibody titers are produced. These Rh IgG antibodies have molecular weight of 160,000 daltons which are able to cross the placental barrier via receptors for the IgFc portion located on synciotrophoblast and coat Rh D positive fetal red cells thus causing their sequestration. IgG sensitized red cells bind via the Fc part of the antibody molecule to FcY receptor (FcYR) on the mononuclear phagocytes in the fetal reticuloendothelial system and thus triggers its cytolysis (18). FcYR mediated phagocytosis by monocytes and macrophages are well developed by early second trimester at 17-18 weeks (19). Sensitized mothers carrying Rh D positive anemic fetus demonstrate high levels of IgG1 antibodies compared to control pregnancies. In addition, there is preferred accumulation of IgG1 in those fetuses affected by hemolytic process along with low levels of fetal IgG3 than are found in fetuses not at risk of HDFN. The IgG3 subclass antibody has a higher potential for inducing phagocytosis and monocyte adherence in-vitro than IgG1. However, IgG1 has a greater influence on the severity of HDFN in-vivo than IgG3 (20).

In the absence of anti-D prophylaxis programme, approximately 1% of Rh D negative women will have detectable anti-D antibodies in their serum by the end of their first pregnancy with Rh D positive fetus. A further 7 - 9% of these women will have detectable antibodies by six months following delivery and roughly the same number will develop antibodies during the second pregnancy with Rh D positive fetus. Thus around 17% of Rh D negative women get alloimmunized by a single Rh D positive fetus (21). The risk of Rh D alloimmunization during pregnancy increases with advancing gestation due to increase incidence of fetomaternal transplacental hemorrhage (15).

All Rh D negative women do not get alloimmunized with Rh D positive fetus in absence of anti-D prophylaxis. Some Rh D negative women are poor responders to Rh D antigen while others may produce anti-D IgG of low potency and efficacy at mediating FcY receptor interaction with phagocytic cells. After maternal alloimmunization to Rh D antigen it is estimated that only 50% of the affected fetus will have mild to no anemia, 25 to 30% will have moderate anemia that will require treatment in the neonatal period and the rest 20 – 25% will develop severe anemia and hydrops. These severely anemic fetuses likely have intrauterine fetal demise or neonatal death unless antenatal fetal therapy is initiated at a specialized center (22).

Hydrops Fetalis

Ascitis (Fig. 1), placentomegaly (Fig. 2), pericardial effusion and subcutaneous oedema (Fig. 3) are the classical features of fetal hydrops on ultrasonography and can also be seen in the neonate if delivered with hydrops (Fig. 4). The affected fetus becomes progressively anemic due to red cell hemolysis and compensates with cardiovascular adjustments and increasing hematopoeisis. Initially to maintain tissue oxygenation cardiac output is increased rather than fetal heart rate due to decrease in blood viscosity and fall in peripheral resistance secondary to vasodilation. In middle cerebral artery mean blood flow velocities due to fetal hypoxemia and anemia have hyperdynamic circulation primarily as a consequence of decreased blood viscosity (23).

Fetal hemoglobin concentration rises with gestational age and hydrops tends to occur at higher hemoglobin levels at later gestation compared to early gestational age (24). It is, therefore more appropriate to relate severity of fetal disease to the hemoglobin deficit for the gestational age. The presence of hydrops indicates fetal hemoglobin deficit of more than 7 g/dl for the gestational age. This means that fetal hemoglobin in the second trimester is less than 4

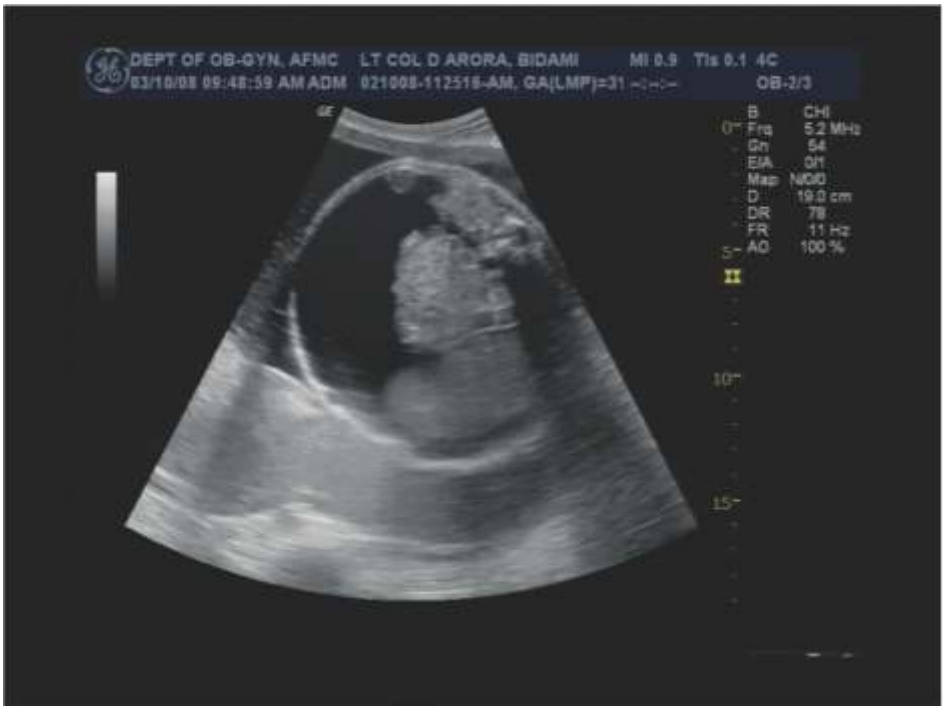

Fig. 1: Gross ascitis in severely hydropic fetus in transverse section seen with floating bowel loops.

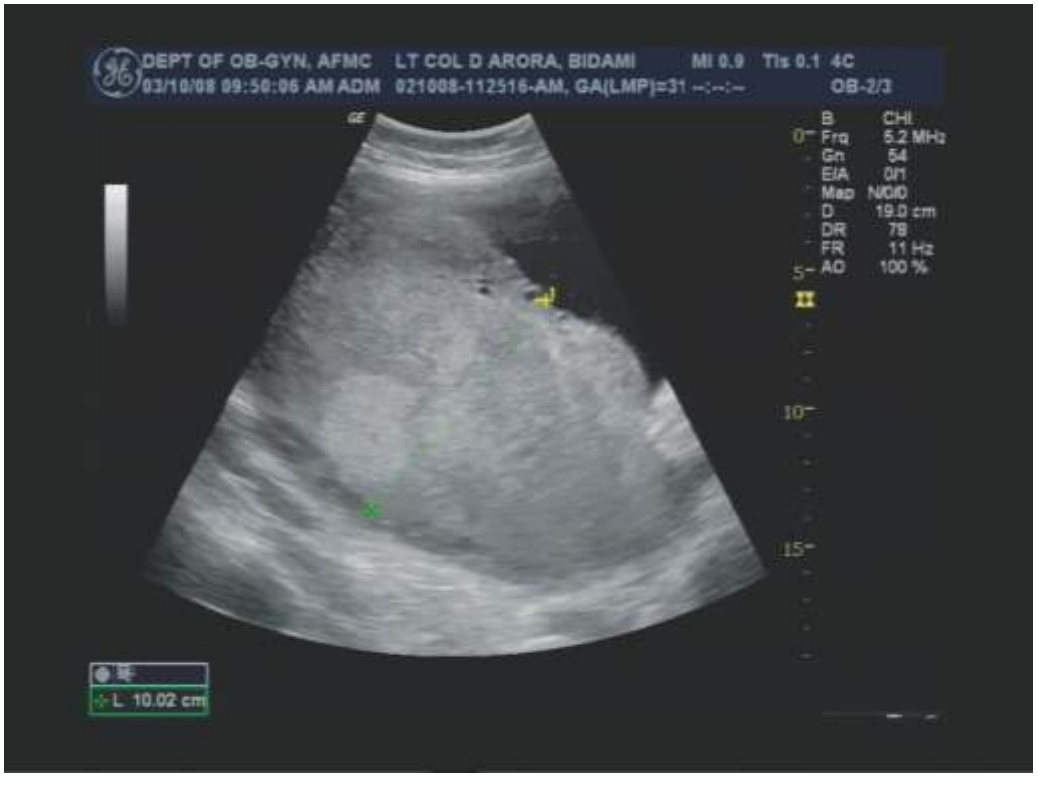

Fig. 2: Placentomegaly in fetus with gross fetal hydrops

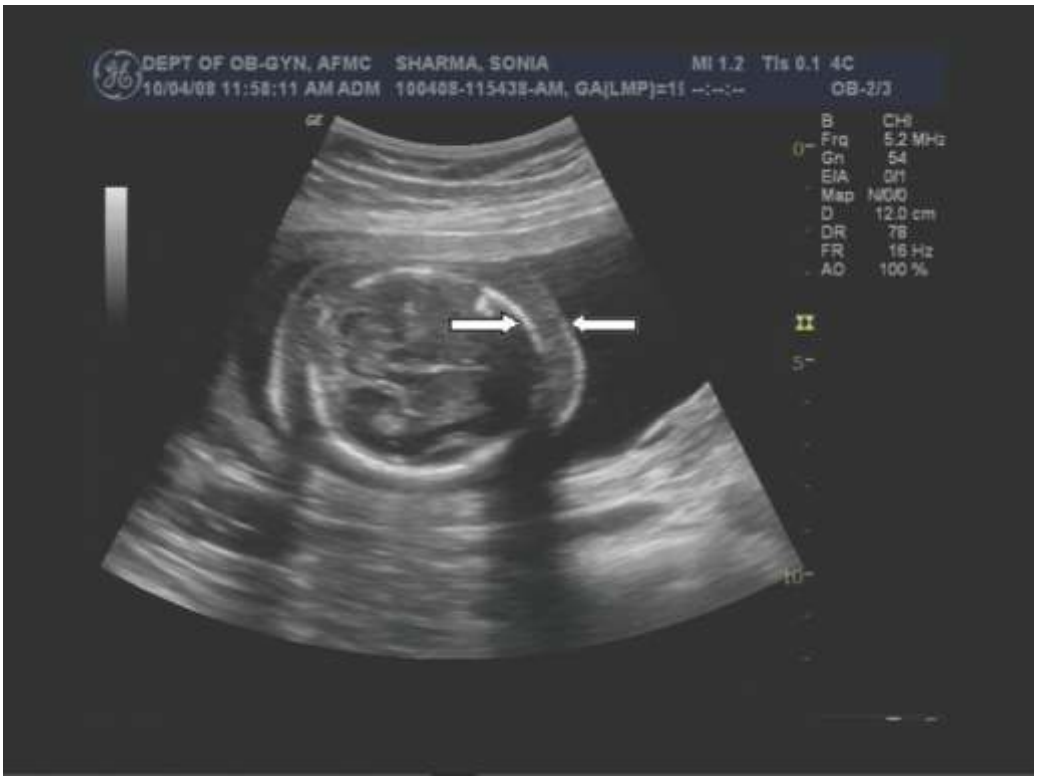

Fig. 3: Scalp edema on ultrasound seen between the arrows in severe hydrops popularly known as 'Buddha Sign' on X-ray of such fetuses

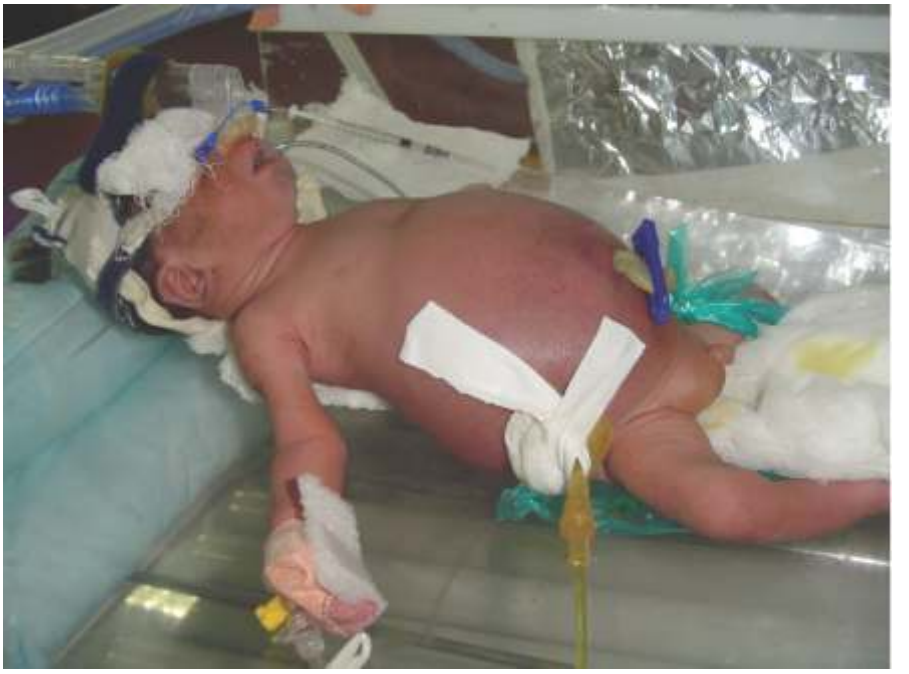

Fig. 4: Hydropic baby being nursed in neonatal intensive care unit. The two-day old baby has gross ascitis with secondary hydrocele clearly seen in this picture. The ascitic fluid is being drained by continuous peritoneal drain placed in-situ.

g/dl or hematocrit less than 15%. However, at these low levels of hemoglobin fetus may not become hydropic. There are several possible explanations for the genesis of hydrops at this degree of anemia like cardiac failure due to myocardial dysfunction caused by fetal metabolic lactacidosis and increased capillary permeability caused by chronic tissue hypoxia (25). In addition iron overload resulting due to ongoing red blood cell hemolysis leads to free iron radical formation and contribute to endothelial dysfunction. Hypervolemia is also considered a reason for fetal hydrops related to a high output cardiac failure that can cause extravasation of fluids in tissues (26). Very high levels of Atrial Natriuretic Peptide are found in such hydropic fetuses implying expanded intravascular volume. Increased amniotic fluid volume appears before the onset of hydrops as normoxemic severely anemic fetuses is able to compensate by an increase in urine production (27).

Routine Rh Screening and Obstetric History

A blood sample must be collected from every woman at her first antenatal visit for ABO and Rh typing for any order of pregnancy. Rh D negative women and those having partial or weak 'D' antigens, were screened for the presence of anti-D in their serum by the indirect antiglobulin (Coombs) test. Paternal blood group and Rh testing should be the integral part of screening. The reduction of maternal surveillance after the father of the baby proves to be Rh-negative is useful unless there is a doubt regarding paternity. In the Rh-negative non-sensitized mother the indirect antiglobulin test should be repeated every four weeks and thereafter till delivery. In Rh alloimmunized mother Rh antibody titer and quantization are the tests employed for estimation of Rh antibodies in maternal serum. A rise in Rh antibody titer indicates Rh D positive fetus.

Screening for antibodies in the second half of the non-alloimmunized pregnancy is very important because it is the most likely time for Rh immunization secondary to transplacental hemorrhage. If in a non-alloimmunized pregnancy antepartum Rh-immune prophylaxis is administered at 28 weeks, a single anti-D titre at 35 weeks is helpful. A titre of Rh-antiglobulin in dilution of 1:8 or greater suggests active immunization (28).

Rh Titer

Several techniques are available to detect Rh antibody titer in maternal serum. The most reliable and sensitive technique is by Indirect Antiglobulin Test (Indirect Coombs Test) which is based on detection of IgG anti-D molecules of maternal serum on 'O' Rh D positive red cells, using a broad spectrum anti-human globulin (AHG) reagent. This test is method of choice in most of the centers for prediction of severity of Rh disease.

The detection of antibody necessitates determining the strength of the immunization by an antibody titer, which is estimated by serial dilutions. The degree of immunization corresponds well to the severity of fetal affection. However, a problem has developed with antibody titers today. Rh immunization has become so infrequent that some laboratories are no longer proficient in the technique. The laboratories thus capable of estimating accurate and reproducible titers play a very useful role in the management of the Rh alloimmunized patients. Laboratories have a critical titer at which there is a significant risk of fetal hydrops mostly between 1:8 and 1:32 dilutions. The absence of antibodies on the initial visit in the patient and subsequent detection on follow-up, groups this patient into the first immunized pregnancy. The alloimmunization of a patient by a prior pregnancy, groups this patient into the subsequent immunized pregnancy. Fetal death and hydrops due to Rh alloimmunization occurs very rarely before 18 weeks. If hydrops has occurred in one pregnancy, then it is likely to re-occur in subsequent pregnancies carrying an Rh-incompatible fetus if treatment by fetal blood transfusion is not initiated (29).

Rh Typing of Fetus

Prenatal DNA based Rh typing of the fetus could show positive results as early as 9 weeks of gestation by obtaining trophoblasts from endocervical canal. Amniotic fluid cells are also be used for this purpose obtained from amniocentesis undertaken as early as 15 weeks. Efforts are now being made to obtain DNA from fetal cells in the maternal peripheral circulation to establish a non-invasive technique that will be available for clinical use in near future (30).

Factors Influencing Rh D Immunization

Besides the use of anti-D IgG several other factors determine the severity of the Rh disease. The D antigenic response is necessary for stimulation of anti-D production. Since D antigen exists only on human RBCs, exposure of Rh D positive blood cells is a prerequisite for sensitization. Rh(D) incompatible transfusion and Rh D incompatible pregnancy are the main sources of D antigen stimulation (31).

Fetomaternal hemorrhage (FMH) is common in third trimester (15) and occurs in about 7% of pregnant women. Complicated and instrumental deliveries increase the risk of FMH. Rh(D) antigen is present on the RBC of a 38 days old fetus. Therefore, the FMH in first trimester spontaneous abortion and induced abortion warrants anti-D IgG prophylaxis. Studies have shown that 99.2-99.3% of women have a FMH less than 4ml at delivery. Up to 50% of larger FMHs occur after normal deliveries (32). However, the following clinical circumstances are more likely to be associated with large FMH:

- traumatic deliveries including caesarean section
- manual removal of the placenta
- stillbirths and intrauterine deaths
- abdominal trauma during the third trimester
- twin pregnancies (at delivery)
- unexplained hydrops fetalis

Tests to estimate the size of the FMH are recommended in many countries including the UK, the USA, Canada, France and Ireland, although not in most European countries. Acid elution technique which detects fetal haemoglobin (HbF) to measure FMH was reported by Kleihauer et al (33). It has been modified by Nierhaus and Betke (34). A thin blood smear prepared from maternal blood collected with ethylenediamine tetra acetic acid (ETDA) is treated with acid hematoxylin to elute maternal hemoglobin and to stain lymphocytes, which may otherwise be identified as fetal cells. The smear is then counterstained by erythrocin to stain fetal red cells. Under light microscope, the number of fetal cells per 10,000 maternal ghost cells is counted. The amount of fetal blood in maternal circulation is calculated by this relationship. The presence of upto four fetal RBC per 10,000 maternal cells signifies 0.15 ml of FMH.

In some European countries (exceptions include the UK, France and Ireland) a standard postnatal dose of 200 µg – 300 µg (1000-1500 IU) is used with no requirement for a routine Kleihauer test (35). In India since the standard dose of anti-D Ig is 300 µg (1500 IU) that is sufficient to clear about 15 ml of FMH, so routine Kleihauer test is not advised. Unfortunately, this policy does not take account of the fact that up to 0.3% of women have a FMH greater than 15ml that will not be covered by 300 µg (1500 IU) of anti-D Ig. The test is also required when massive FMH is suspected. In UK if the 1500 IU dose is implemented without a test to quantitate FMH, over 200 women each year will receive less protection than they do now (36). The recommended policy in the UK is to obtain an anticoagulated blood sample as soon as possible (within two hours) after delivery and to undertake a Kleihauer screening test to identify women with a large FMH who need additional anti-D Ig.

While the Kleihauer acid elution test is the test usually undertaken in the UK and Canada, tests that specifically identify Rh D positive red

cells are used in the USA. Flow cytometry offers an alternative technique for quantifying the size of FMH (37). It has a number of advantages in that results are more accurate and more reproducible than those from the Kleihauer test and that it detects Rh D positive cells, making it particularly helpful in patients with high HbF levels. Not all hospitals will have ready access to a flow cytometer though several Blood Centres offer to estimate FMH. Flow cytometry is probably most effectively employed in those cases where a Kleihauer screening test indicates a large FMH that requires accurate quantitation and follow-up. The direct flow cytometry method uses fluorescent isothiocynate (FITC) or phycoerythrin (PE) labeled monoclonal anti-D. In the indirect method anti-human globulin reagent labeled with FITC or PE is employed. Chapman et al (38) has recommended flow cytometric technique for confirmation of amount of FMH when the leak is greater than 4 ml. The rosetting technique1 is a relatively simple serological method that offers another alternative for quantifying FMH of Rh(D) positive red cells greater than 4ml.

Effect of ABO Incompatibility

An ABO incompatible pregnancy offers protection against Rh alloimmunization, as A or B group fetal red cells are destroyed by anti-A or anti-B antibodies in the maternal circulation before Rh D can be recognized by the maternal immune system. Finn *et al* found that these feto-maternal microtransfusions were much less likely to occur in the case of women who were bearing an ABO incompatible baby (8). This reduces the chance for maternal sensitization by 20% (39). However, vice versa, alloimmunization capability is enhanced if the Rh positive fetus is ABO compatible with Rh D negative mother due to delayed destruction of fetal red cells by maternal immune system.

Immune Response of D antigen

It is surprising to note that iso-immunization does not occur in every case which an Rh D negative woman bears an Rh D positive child, but the phenomenon is doubtless dependent on the size, quantity or repetition of the antigenic stimulus. Once the mother has become immunized, her serum will contain antibodies for most of the rest of her life. Immune response to D antigen is genetically controlled. About one third Rh D negative women do respond to D antigen. The remaining two third women are either good or poor responders. Poor responders require several D antigenic stimuli before they produce anti-D Ig. The immunogenicity also depends on the number of D antigenic sites per RBC (31). It follows therefore that if iso-immunization has occurred as a result of pregnancy, the results are more likely to appear, not in that pregnancy but in the subsequent ones on disruption of placental continuity that provokes sensitization in susceptible women by secondary immune response.

Rh Status of Father

All children of Rh D negative mothers will be Rh D positive if the father is homozygous Rh D positive (D/D) but 50% children will be Rh D negative if the father is heterozygous (D/-) positive. If both the parents are Rh D negative, then the children will always be Rh D negative (40). Determination of definite Rh genotype is difficult, as anti-D antibody does not exist. With the help of anti-C, anti-c, anti-D, anti-E and anti-E reagents, the Rh phenotype can easily be identified.

Other Prenatal Investigations

Ultrasound assessment of at-risk fetus is mandatory for detecting fetal hydrops. Weekly assessment of high-risk patients is appropriate. The sonographic features of hydrops fetalis include ascites, pericardial and pleural effusion, subcutaneous and scalp edema, polyhydramnios and placentomegaly. However, only two-thirds of fetuses with such low hemoglobin demonstrate fetal ascitis. The earliest sonographic feature of hemolytic disease has

been said to be enlargement of the heart and in particular reference to the right atrium (29).

Doppler studies in the evaluation of severe alloimmunization are performed in the view of correlation between decrease in fetal hemoglobin and increased maximum systolic velocities in various fetal blood vessels as a consequence to decrease in blood viscosity and increase in cardiac output. One of the most significant breakthroughs in recent years has been the research that validates the Peak Systolic Velocity Middle Cerebral Artery (MCA-PSV) as a reliable screening tool to detect fetal anemia (Fig. 5). Vyas and coworkers (1990) were the first to report the use of the Doppler velocity in the MCA to detect fetal anemia (41). This method is based on the fact that anemic fetuses have an increased blood flow velocity due to high hemodynamic circulation. Mari and co-workers (2000) generated the normative data for gestational age using threshold value of 1.5 Multiples of Median (MoM) for MCA-PSV to predict moderate to severe anemia. Doppler measurement of the MCA-PSV is performed (42) in recumbent position. The fetal vertex is visualized, and an axial plane that included the thalami and cavum septum pellucidum is obtained. The transducer is moved caudally until the circle of Willis is in view with color flow Doppler imaging. The MCA closest to the maternal skin is identified, and the angle of the ultrasound beam to the MCA blood flow is positioned as close to zero degrees as possible. The velocity measurement is made as close to the MCA origin from the circle of Willis as possible. MCA-PSV measurements are taken during periods of fetal quiescence (absent fetal breathing motion or fetal movements). The peak of the velocity waveform is measured. Multiple measurements are obtained during each ultrasound examination. The highest value, obtained with an angle of insonation as close to zero as possible, is recorded as the PSV measurement. If the MCA-PSV measures greater than 1.5 MoMs for gestational age, then the pregnancy is considered to be at risk of significant fetal anemia (Fig. 6). It has a positive predictive rate for moderate to severe fetal anemia of 74% with a 10% false positive rate.

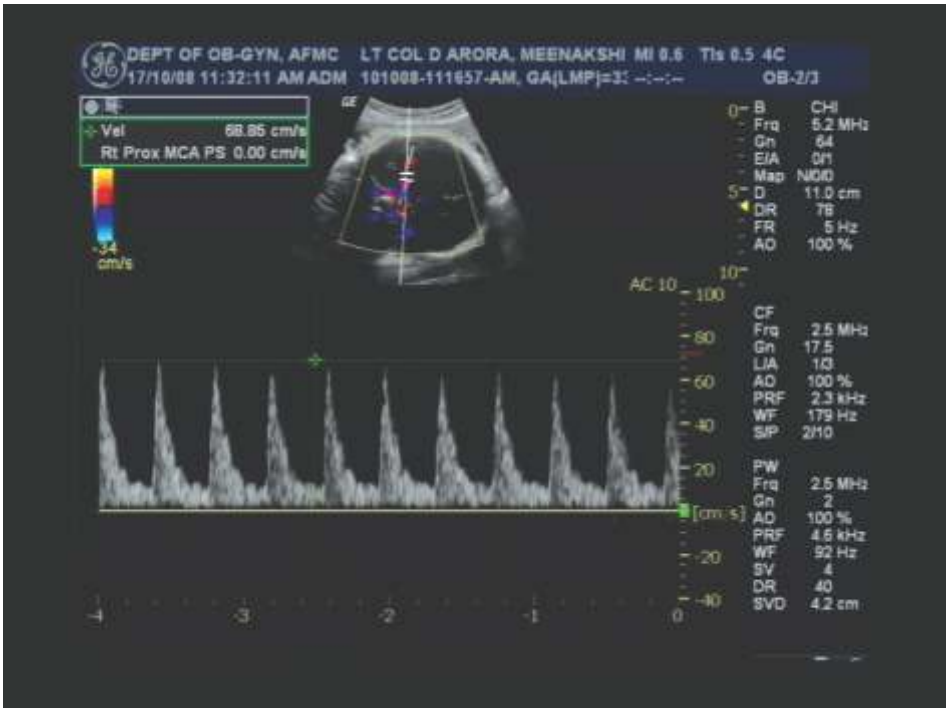

Fig. 5: Middle cerebral artery-peak systolic velocity measurement at origin from circle of Willis.

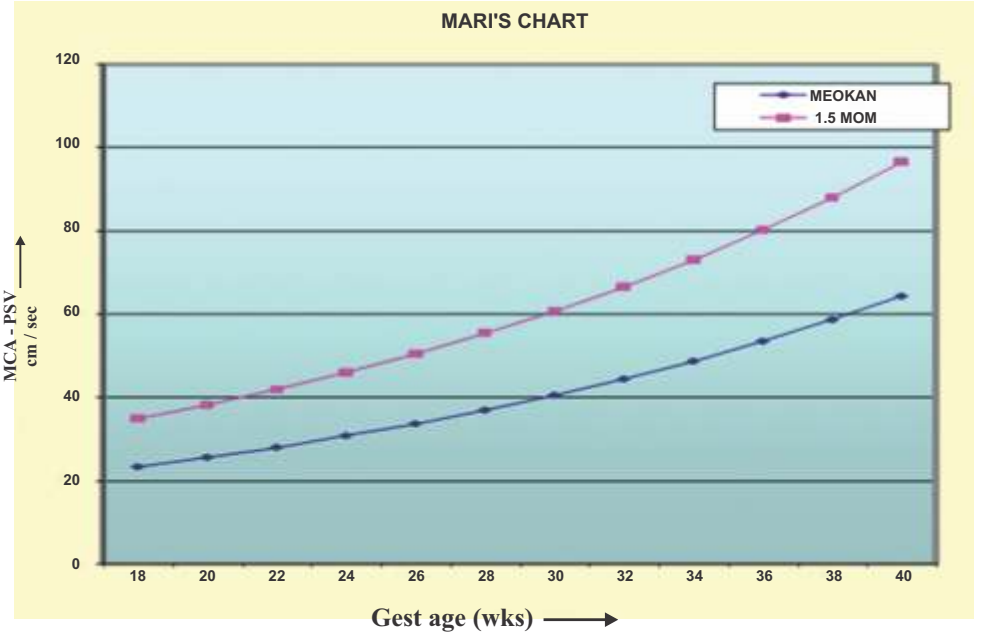

Fig. 6: The Mari *et al* (42) chart developed to show an association between Middle Cerebral Artery-Peak Systolic Velocity in reference to gestational age. The graph line marked with filled rectangles marks the cut-off value in 1.5 MoMs of MCA-PSV for the gestational age.

MCA Dopplers can be started as early as 15 weeks gestation but are not reliable after 35 weeks. The study of MCA-PSV is initiated whenever the antibody Coombs' titre rises beyond the critical value while on regular antenatal follow-up. The advantage of serial MCA measurements is that they reduce the need for invasive diagnostic procedures like amniocentesis and cordocentesis by more than 70% (42). Doppler measurement of the MCA-PCV can safely replace invasive testing in the management of Rh-alloimmunized pregnancies (43).

Invasive Testing of the Fetus

Pregnancies complicated by Rh alloimmunization have been evaluated to assess degree of fetal anemia indirectly with the use of serial invasive amniocentesis to determine bilirubin levels as a result of fetal hemolysis by measuring in the amniotic fluid, the change in optical density at a wavelength of 450 nm (ΔOD_{450}) by spectrophotometric analysis; however, this procedure carries risks. Nicolaides et al have shown that the backward extrapolation of the Liley's chart lines is of little value in determining severity of fetal anemia before 27 weeks gestation. The wide scatter and fluctuation of ΔOD_{450} values leads to a 68% false negative rate for severely affected fetuses between 18 and 25 weeks gestation. Doppler ultrasonography of middle cerebral artery has emerged as more sensitive and more accurate (95 percent confidence interval) than measurement of amniotic-fluid ΔOD_{450} (29). The other method of assessing the degree of fetal anemia is directly by hematological studies on fetal blood sample. The indication for the invasive testing should be decided on:

- Previous obstetric history
- Duration of pregnancy
- Maternal antibody levels
- Ultrasonography
- Doppler studies with MCA-PSV >1.5 MoMs for the gestational age

The aim of management in Rh alloimmunized pregnancy is to allow the pregnancy to continue to a safe gestational age,

ideally 37 completed weeks or more. The fetus at the time of delivery should not be hydropic or severely anemic for a favourable outcome. This specialized management requires expertise and referral to a fetal medicine centre once invasive testing is being contemplated. In the present scenario there has been a decline in number of amniocentesis for the favour of non-invasive assessment and fetal blood sampling (43).

Cordocentesis was introduced in the mid 1980s. The direct access to the umbilical cord vessels by ultrasound guided needle puncture allows clinicians to measure fetal hematocrit, reticulocyte count, bilirubin level and a direct Coombs test. However, cordocentesis as a primary surveillance tool is not recommended due to 1% risk of fetal loss and chance of fetomaternal hemorrhage enhance maternal sensitization. Presently, cordocentesis is reserved as a second line diagnostic tool once amniocentesis or MCA Doppler suggests fetal anemia. Conventionally, serial amniocenteses were performed for spectrophotometric studies to detect fetal bilirubin by ΔOD450 for estimating fetal anemia based on the Liley's reference chart. Serial peak middle cerebral artery velocities using Doppler ultrasound have now become the mainstay in these pregnancies to screen for fetal anemia (42). In anemic fetuses remote from term, intrauterine fetal blood transfusion is usually necessary either intravenously, through ultrasound-directed puncture of the umbilical vein with the direct intravascular or intra-peritoneal injection of red cells. In intra-peritoneal transfusion the transfused blood is deposited in the fetal peritoneal cavity. Perinatal survival rates of more than 90% have been reported in fetuses thus managed. Development of hydrops fetalis, however, has been reported to reduce the chance for a favourable outcome by up to 25%. Long-term studies have revealed normal neurological outcomes in more than 90% of such anemic fetuses treated with fetal blood transfusion.

Fetal Blood Transfusion

Delivery before 34 weeks in a severly anemic fetus is so readily rewarded by a neonatal death in olden days that every attempt was made to secure degree of maturity but most severely affected babies died in-utero often prematurely. In order to stave off intra-uterine death, intrauterine fetal transfusion (IUT) was introduced by Dr Liley in 1963 that provided hope to salvage these desperate cases (5). This procedure captured the imagination of the whole obstetrical world as it represented the first successful attempt at directly correcting a fetal disorder before birth. The procedure of intra uterine transfusion has been refined over the years from intra-peritoneal to intravascular or intra-cardiac depending on the clinical scenario.

Serial ultrasound examinations for MCA-PSV measurement are performed every 1 to 3 weeks depending on the antibody titer. Evidence of evolving fetal hydrops is evaluated at each ultrasound examination. If the MCA-PSV measured greater than 1.5 MoMs for gestational age, then the pregnancies are considered to be at risk of significant fetal anemia and are offered fetal blood sampling (42). Intra-uterine fetal blood transfusion is performed whenever fetal hemoglobin is less than 9 g% or hematocrit is less than 30% for the fetuses with gestational age less than 34 weeks (44). If the gestational age is more than 34-35 weeks, delivery is considered in view of expected fetal lung maturity. However steroids for fetal lung maturity should be administered before 34 weeks on regular based protocol in view of contemplating early delivery secondary to complication by fetal intervention. The pregnancy can be prolonged upto 35-36 weeks in absence of fetal anemia as predictability of MCA-PSV for fetal anemia decreases after 35 weeks of gestation (42).

Fetal surveillance following fetal blood transfusion is done by monitoring fetal heart on cardiotocography for about one hour after the procedure. The fetal heart variability observed during this period is often poor due to effect of

fetal paralysis (45). The mother is advised to monitor daily fetal movement count and attend to bi-weekly to weekly monitoring by ultrasonography to evaluate MCA-PSV and umbilical artery velocimetry until next fetal blood transfusion.

After-effect of Fetal Blood Transfusion

After intra-uterine transfusion the fetal circulation will contain adult red blood cells and usually after three transfusions the entire fetal circulation contains no fetal red blood cells. Fetal erythropoiesis gets fully suppressed and fetal blood group at birth is 'O' Rh D negative. It can take several weeks for complete resumption of the neonatal erythropoiesis and top-up blood transfusions may be necessary as the baby develops anemia with growth. Regular monthly hemoglobin of such babies is advocated upto 6 months of age. However in refractory babies with absence of erythropoiesis, erythropoietin may prove to be efficacious. However, such babies born after being treated with intra-uterine blood transfusion show normal growth and neurological development (46, 47). Rh alloimmunized fetuses show high serum ferritin levels and repeated intra-uterine transfusions are further associated with iron overload in such babies. It is recommended that serum ferritin levels are monitored in such babies and iron supplementation withheld until its levels are in normal range (48).

Intra-peritoneal Fetal Blood Transfusion (IPT)

Sir William Liley successfully pioneered this procedure more than 40 years ago. The principle of intra-uterine fetal blood transfusion was based upon the fact that the fetus very readily absorbs blood directly from the peritoneal cavity far more readily than adult. The post mortem evidence indicates that the blood is completely absorbed within ten days of the IPT or earlier. However in the present day practice intra vascular route has been advocated the treatment of choice. IPT relies on placing the donor red cells into the peritoneal cavity so that they are absorbed into the fetal circulation via subdiaphramatic lymphatics and thoracic duct. The presence of ascitis in hydropic fetus reduces the efficacy of this procedure and increases the intra-peritoneal pressure in fetal abdomen that compromises venous return to fetal heart and cause bradycardia. Intravascular fetal blood transfusion (IVT) is considerably more successful in reverting the hydrops and ensuring survival in the neonatal period (49). The volume of blood transfused in IPT is dependent on empirical formulas as pre- and post-transfusion hemoglobin values for calculating amount of blood to be transfused is not available. In a specific clinical scenario IPT is the method of choice if treatment becomes necessary at very early gestation of 18 weeks or under when direct access to fetal vasculature is difficult and hazardous.

Technique of IPT involves placement of 18-20 gauge spinal needle into the fetal abdomen under ultrasound guidance. Ideally, the needle should enter the fetal abdominal cavity through the anterior abdominal wall, below the umbilical vein and above the fetal urinary bladder. This avoids trauma to the fetal liver and other fetal intra-abdominal organs. To verify that the needle is correctly placed the operator should either aspirate the ascitic fluid in hydropic fetus or in absence of ascitis, can infuse bolus of saline solution in the peritoneal cavity while observing the tip of the needle sonographically for confirming correct placement. Dr. Liley during his initial fetal transfusion via peritoneal route used to perform under fluoroscopic control (5). He used to confirm the correct needle placement by injecting radio-opaque dye in the fetal abdomen while observing fetal bowel loops displacement under fluroscopic control (Personal communication to author from Prof. Anjanellu). The needle can then be connected via three-way to the infusing tubing of closed circuit as described for IVT. Throughout the transfusion the tip of the needle is monitored sonographically to observe the flow of blood entering the peritoneal cavity and the fetal heart

rate observed. The appearance of persistent bradycardia indicates that the IPT should be discontinued (29). The amount of donor blood to be given is calculated using the following empirical formula that calculates according to the gestational age of the fetus rather than the degree of anemia:

Volume of Donor Blood Required for Transfusion (ml)= (Gestation period in weeks – 20) X 10 ml

Intra Vascular Transfusion (IVT)

The direct IVT was introduced in mid 1980s. Till then IPT was the route of choice since Dr Liley introduced it in early 1960s. Experience in hydropic fetuses indicates that absorption from the peritoneal cavity is compromised. Harman et al divided fetuses into hydropic and non-hydropic group at time of first transfusion. He found a 13% increase in survival of non-hydropic fetuses using IVT as compared to IPT. In hydropic fetuses the rate of survival almost doubled with IVT. IVT also decreases the incidence of neonatal exchange transfusion and shortens the stay in neonatal intensive care unit (50). The IPT route still has a place to deliver red blood cells to the non-hydropic fetus when it is difficult to access the umbilical vein route either through the umbilical cord or the intra-hepatic part.

The blood used for fetal blood transfusion is adult group 'O' Rh D negative blood that should ideally be not more than 72 hours old and cross-matched with the maternal blood. It should be screened for hepatitis B and C, cytomegalovirus and HIV. To prevent graft-versus-host like complications in the fetus, the donor blood should be passed through leucocyte depletion filters followed by irradiation to remove the white blood cells. The donor blood cells are ideally packed to a hematocrit of 75 – 85% to minimize the volume of transfused blood.

Technique of intra-uterine fetal blood given by IVT involves insertion of a long spinal needle of 20-22 gauge (depending on period of gestation) under ultrasound guidance in the umbilical vein to obtain fetal blood sample followed by transfusion. The operator obtains the fetal venous access by a free hand technique or with needle guide attached with ultrasound transducer. Three trained persons are required for this procedure: an experienced operator who performs the cannulation of fetal umbilical vein and monitors the transfusion throughout by sonographic visualization, an assistant who administers blood and a third person assists in performing the blood tests and calculations. Tocolysis and suitable antibiotics may or may

Fig. 7: Fetal blood transfusion in progress under ultrasound guidance with blood being transfused through a closed circuit with free hand technique.

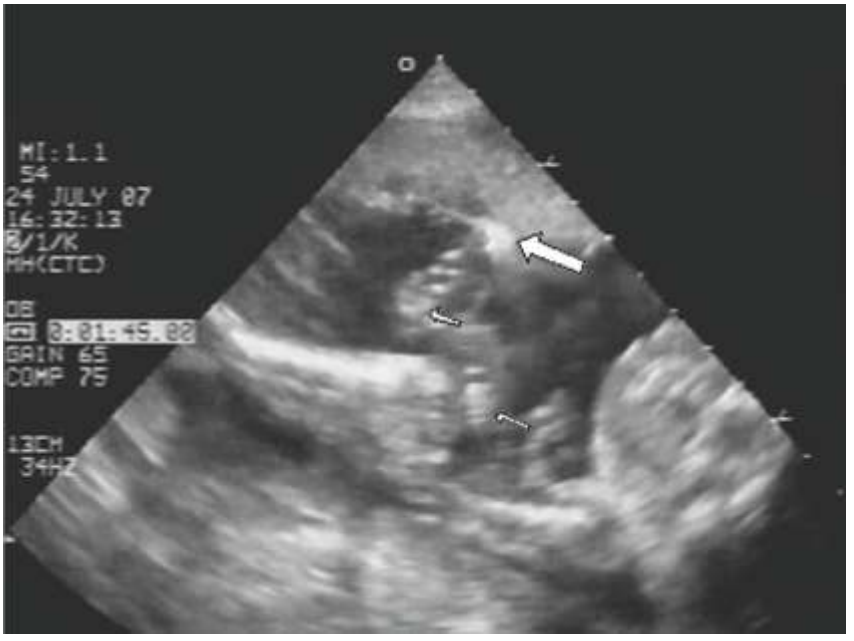

Fig. 8: The umbilical vein is infused with concentrated donor red cells, producing turbulent cascade during infusion that stops immediately when the infusion stops. The thick arrow shows the needle tip and thin arrows show the turbulence inside the umbilical vein.

not be administered before this invasive procedure.

The umbilical vein at its placental insertion site is used for transfusion (Fig. 7 & 8). Frequently, placental position and fetal lie prevents safe access to the site of placental insertion of umbilical cord for transfusion. The alternative approach is to cannulate the umbilical vein in the free loop of umbilical cord or intra-hepatic part of umbilical vein that requires piercing the fetal abdomen with fetal paralysis with pancuronium. However, some workers do suggest universal fetal paralysis for the procedure by any vascular approach as fetal movements can be detrimental even after a safe vascular access (51). In my experience I have found that universal fetal paralysis is beneficial as any fetal movement may lacerate the vessel or dislodge the needle even if umbilical vein is approached in anterior placenta at insertion. Pancuronium is given to the fetus intra-muscularly or intra-vascularly (approximately 0.3 mg per kg of the estimated fetal weight) with ultrasonography guidance and has a theoretical advantage of increasing fetal heart rate secondary to catecholamine release. This helps in maintaining the fetal heart rate at a pre-transfusion rate and prevents fetal bradycardia during the procedure.

Once the access to the fetal circulation is obtained, one ml sample of fetal blood is drawn for determining fetal hematocrit and blood group. A fetal hematocrit less than 30% or below 2 SD for the gestational age, is considered an indication for in-utero transfusion at the same sitting (52). The volume of blood transfusion is determined by pre-transfusion fetal hematocrit, the estimated fetoplacental blood volume for the gestational age and the hematocrit of the donor blood. Nomograms have been worked out based on these parameters to provide an estimation of volume of donor blood required to raise the fetal hematocrit to 40% (53). The volume of red cell in milliliters can be calculated by Mandelbrot method using the formula given below (54):

$$V\ (\text{Volume})_{\text{transfused}} = \frac{V_{\text{fetoplacental}} \times (\text{Hematocrit}_{\text{final}} - \text{Hematocrit}_{\text{initial}})}{\text{Hematocrit}_{\text{transfused blood}}}$$

At the end of the procedure 1 ml of fetal blood is aspirated to estimate post-transfusion fetal hematocrit. The blood is transfused at a rate of 10 ml per minute in 5-10 ml aliquots. During the procedure the flow of blood as a cascade is continuously visualized on the ultrasound screen that confirms correct needle placement. The fetal heart rate is periodically checked to look for fetal bradycardia. The onset of fetal bradycardia warrants abandoning of the procedure to prevent cardiac overload and arrest. A free-hand technique of fetal venous access allows independent movement of the ultrasound transducer for the periodic visualization of the fetal heart during the transfusion for monitoring. This free hand technique is preferred by most workers but requires high amount of expertise. However, the use of needle guide with the ultrasound probe has a better safety profile with less expertise.

Top up transfusions compared to exchange transfusions are quicker and reduce the risk of needle displacement, bacterial contamination and umbilical vein thrombosis. The second transfusion should not be performed later than 2 weeks after the first transfusion. In cases of severe fetal anemia and grossly hydropic fetuses or in cases where the first transfusion was small, the second transfusion may be required after a week. The mean fall of hematocrit is around 1% per day but it can have wide variation between the first and second transfusion. The fetal loss rate during the procedure ranges from 4 to 14%. The risk is more if fetal blood transfusion is given before 20 weeks period of gestation (29). Transient fetal bradycardia is the most common complication and it occurs in around 8% of the cases. The potentially fatal complications for the fetus are cord accidents like cord hematoma, umbilical artery spasm, hemorrhage from the cannulation site, thromboembolism and overloading of fetal circulation. Worsening degree of maternal sensitization, chorioamnionitis, premature rupture of membranes and preterm labour are also possible complications of this invasive procedure (55). Graft-versus- host-reaction can take place if the transfused blood is not leukocyte depleted before transfusion.

Combined IVT and IPT

A combined IVT – IPT procedure results in a more stable fetal hematocrit between the two fetal blood transfusions (56). The hypothesis is that initially IVT is performed to achieve a final hematocrit of 40% followed by intraperitoneal infusion of donor blood which serves as a reservoir by allowing slow absorption of red cells between the procedures and allowing more stable hematocrit.

The technique used is the same for IVT. An adequate amount of donor blood is given intra-vascular to raise the fetal hematocrit to around 40%, as confirmed by a post transfusion hematocrit. The same spinal needle is then used to perform an IPT. This combined procedure becomes particularly easy if intra-hepatic part of umbilical vein is used for IVT (57). The amount of donor blood used in IPT should be the same that would be required intravascularly to raise the hematocrit to 60%. If a large amount of ascitic fluid is present in a hydropic baby then before IPT it should be removed.

Although the combined procedure has a theoretical advantage but most centers world over exclusively perform only IVT. The combined procedure warrants two needle punctures with a prolonged fetal procedure time that is considered as a disadvantage. The experience of fetal blood transfusions in twins is limited. There is a need to sample each fetus in dizygotic twins for antigen testing and follow-up with MCA Doppler studies. There is a difficulty in identifying corresponding cord insertions in twins that prevents transfusion to the corresponding anemic fetus. In this scenario intra-hepatic portion of umbilical vein may be the preferred target for vascular access. In monozygotic twins though the screening is done as of dizygotic twins but the fetal blood transfusion should be done with caution. The transfusion of one monochorionic twin may

result movement of red blood cells to the other member of monochorionic pregnancy through intraplacental anastomosis. This may result in under or over transfusion of any of the twin (58).

Intra-cardiac Transfusion (ICT)

ICT is advocated in critical situations when severe early disease is present in fetuses. These fetuses when treated with IVT develop exsanguinations due to vessel trauma and presence of possible thrombocytopenia. In such situation the possibility of fetal resuscitation pass in a very short time. The fetal vascular collapse prevents repeating vascular puncture that leads to fetal demise. ICT in such fetuses is life saving. ICT also has an exceptional role of salvaging fetuses with very early hydrops especially less than 16 weeks gestation (59).

Technique of ICT involves use of 20-gauge spinal needle as it is easily maneuvered. The right ventricle of the heart is easily approachable but one can also use the left ventricle. IVT principals of fetal transfusion are followed as discussed. The turbulent cascade is visualized in the umbilical arteries exiting the fetus. The transfused blood should be warmed to normal body temperature before transfusion. Cold blood from the blood bank during ICT produces cardiac slowing and ventricular dysfunction. Although there is a potential risk for pericardial effusion by ICT but it is not common (60).

In my experience of more than 525 fetal transfusions have been performed till date at our center and the intravascular transfusions had been the mainstay of management with an excellent salvage rate of anemic fetuses. The IVT has been performed in a fetus of as early as gestational age of 18 weeks at our centre with a successful outcome. In one fetus of 17 weeks, IPT was performed, as the venous access was not feasible in such a small fetus followed by IVT at subsequent requirement with a success.

Red Cell Transfusion and Component Type

Red cells of O negative group used for IUT are centrifuged to increase the haematocrit to around 80%, leucodepleted and irradiated to prevent transfusion-associated graft-versus-host disease (TA-GvHD) and have specific features. These cells have only a 24-h shelf life following irradiation and the supplying Blood Bank ideally requires a minimum of 24 h notice.

Blood for IUT should not be transfused straight from 4°C storage due to risks of fetal bradycardia but there are no specifically designed warming systems for the small blood volume required and the component should not be exposed to radiant heaters or sunlight as the temperature is unmonitored and there is a risk of haemolysis. Transfusion volume required may be calculated based on donor and fetal haematocrit and the estimated fetoplacental blood volume. The fetoplacental volume depends on gestation age and fetal weight.

In urgent situations, if IUT units are unavailable, acceptable alternatives are irradiated neonatal red cell exchange units or irradiated pediatric packs. These should be available at all time in Blood Banks, so use of non-irradiated blood for IUTs should be extremely rare. In emergency situations where requesting irradiated red cells from the Blood Bank would cause life-threatening delay, it may be necessary to use a non-irradiated alternative, ideally a fresh neonatal pediatric pack (before the end of Day 5 following donation) or an exchange transfusion unit. The risk of TA-GvHD using these alternatives, although not eliminated, is acceptable in an emergency because these components have been leucodepleted and in most cases there will be no shared haplotype between donor and recipient. Maternal blood should not be used for IUTs because of the significant risk of TA-GvHD (61).

If the fetus receives more than three transfusions, fetal circulation will only show the transfused adult blood group 'O' Rh(D) negative.

The fetal erythropoiesis is suppressed and can remain suppressed up to six months postnatally which should be monitored by regular hemoglobin estimation in the neonate. Top up transfusion may be necessary till resumption of erythropoiesis. These neonates do not have increased risk of compromise at birth and attain normal growth. There is also no neurodevelopment abnormality on long term follow-up (62). Other treatment modalities like maternal plasmapheresis or intravenous immunoglobulin (IVIG) administration has become history with the advent of modern fetal blood transfusion techniques. However, IVIG has a potential role in management of Rh alloimmunized neonate in the intensive care unit and also to the fetus while performing intrauterine blood transfusion (63).

Testing the Baby at Birth

As soon as the baby is born, 10 ml of cord blood should be collected in a heparinised tube for hemoglobin estimation, Coombs' testing and baseline bilirubin. The blood can be collected from the placental end of the severed cord but to avoid contamination with Wharton's jelly it should not be squeezed out. A blood smear is also made for detection of immature red cells. The hemoglobin level of the neonate at birth is an important prognostic indicator at birth.

Future Pregnancies

Once a woman has had one affected child by Rh allo-immunization then the outlook for further children is likely to be no better if not treated in-utero. It will also depend more on whether the male partner is homozygous or heterozygous. Unfortunately about three quarters of the fathers of affected children are in fact homozygous making outlook bleak for further child bearing as there is a tendency for the disease to be more severe in each successive instance. IUT, however may be impressive life saving procedure but how much better is to prevent iso-immunization in the first place that I feel should be the moto.

The advancement of performing fetal blood transfusion in the management of fetal disease caused by red cell alloimmunization has meant that sensitized women, who in past had suffered multiple fetal and neonatal losses, can now be optimistic about achieving a successful pregnancy outcome. Fetal blood transfusion is the mainstay of the management of Rh disease with MCA-PSV offering a reliable non-invasive modality for determining the fetal anemic status in such pregnancies. Doppler measurement of the peak velocity of systolic blood flow in the middle cerebral artery can safely replace invasive testing in the management of Rh-alloimmunized pregnancies (64, 65). It guides the timing of IUT, aids in monitoring the post transfusion period and helps in optimizing delivery timing to near term. These measures result in an improved perinatal outcome in pregnancies previously considered unsalvageable. Rh(D)-alloimmunized fetuses with ascites / hydrops at the time of the first transfusion have a survival rate of 87%. Alterations of several biochemical fetal blood indices are present at the first fetal blood sampling / transfusion, but most variables normalize with intravascular transfusions according to latest reports (66). The diagnostic accuracy of noninvasive fetal Rh determination using maternal peripheral blood is 94.8%. Its use can be applicable to Rh prophylaxis and to the management of Rh alloimmunized pregnancies in near future as a routine (67).

Red blood cell alloimmunization in pregnancy continues to occur despite the widespread use of both antenatal and postpartum rhesus immunoglobulin. It is due to inadvertent or inadequate omissions in administration as well as antenatal sensitization prior to rhesus immunoglobulin given at 28 weeks' gestation. The vigorous policy of Rh prophylaxis has reduced the Rh problem to about a tenth of what it was in Dr. Liley's time but it cannot eliminate altogether. Additional instances are attributable to the lack of immune globulins to other (non-D) red cell alloantibodies such as c, Kell and Fy. Since immune-prophylaxis is not available for

non-rhesus-D disease, alloimmunization during pregnancy by these non–D red cell allo-antibodies will continue to occur!

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Spinal TB: Impact of Research Evidence on Clinical Practice

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ABSTRACT

The evidence generated while treating the patients is the key for growth of science. Finding answers to series of research questions spread over many years may change the clinical practice. This presentation is based on 25 research questions, 44 publications while treating 3300 patients over last 28 years (1990-2017) which has substantially changed the objective of treatment in spinal tuberculosis (TB) from healing of lesion with sequelae of spinal deformity and paraplegia to achieving healed status with near normal spine.

Three cases of late-onset paraplegia were evaluated (1990) by newly introduced MRI. The syringohydromyelia and severe cord atrophy were attributed as the cause of paraplegia. We conducted a series of prospective studies to define and correlate MRI observations on spinal cord in paraplegia and followed the treatment outcomes. The cord edema, myelomalacia, cord atrophy and syringomyelia were observed in cases with neural complications. The patients with cord edema and liquid compression are predictor for neural recovery, while dry lesions and myelomalacia for poor neural recovery. The mild cord atrophy was consistent with neural recovery while severe cord atrophy with sequalae of neural deficit. Upto 76% canal encroachment was found compatible with intact neural state. Spinal deformity in TB spine is better prevented than treated. The contagious vertebral body disease with intact disc spaces, subperiosteal and paravertebral, septate abscesses, intra-osseous and intraspinal abscesses are considered features of spinal TB and resolution of abscess and fatty replacement is characteristic of healing. The clinicoradiological predictors for diagnosing spinal TB in predestructive disease were defined. Only 35% patients achieved healed status on MRI by DOTS regimen at 8 months, Hence, it is unscientific to stop antitubercular treatment (ATT) at fixed time schedule. The criteria to suspect multi-drug resistant (MDR)-TB and guide to treatment were defined.

Residual Kyphotic deformity in spine TB produces severe proximal/distal degeneration of spine and/or late-onset paraplegia. We correlated the final kyphosis with initial vertebral body (VB) loss, where 1.5 VB height loss will produce 600 spinal deformity or more, hence surgical correction of spinal deformity is indicated. The surgical steps of kyphotic deformity correction are: anterior corpectomy, posterior column shortening, instrumented stabilization, anterior gap grafting and posterior fusion in a single stage and sequentially. The surgical incision of costo-transversectomy was modified so that kyphosis correction and posterior Hartshill instrumentation can be performed simultaneously. The retroperitoneal extrapleural approach for dorsolumbar spine was described. Meta-analysis of spinal instrumentation in TB spine established the lack of defined indication of instrumented stabilisation. Panvertebral/ long segment disease, kyphotic deformity correction are listed as indications of

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instrumented stabilisation in TB spine. The end point of treatment in spinal TB still eludes us to resolve the optimum duration of ATT regimen. The PET scan may be used to define it. We believe if a clinician works slow and steady on a series of research questions and by sustained focused efforts can change the clinical practice. We after this sustained research work could contribute in framing Bone and Joint TB guidelines and publish as monograph.

Keywords: Bone tuberculosis, TB spine, antitubercular therapy, kyphotic deformity.

Introduction

The medicine has evolved as an art based on experience of observing and treating the persons in pain. In ancient time it was hypothesized that disease occurs due to curse of supernatural forces. Gradually over the years since (3000 years) ago a scientific basis of disease was found to execute treatment predictably. The persons involved in executing treatment remedies developed treatment practices over centuries by continued observations. They transmitted this experience by passing them to their disciples to cultivate expertise. Currently the treatment practices are not based on expert opinion. It is based on evidence generated by researches conducted over a series of similar patients in different parts of the world and published for scientific scrutiny and its continued validation.

The human body is complicated, where the biological processes (physiological and pathological) are affected by multiple factors. The response of each human body to inciting agents to cause disease and the response to treatment is variable. That is why suspecting a diagnosis from the symptomatology, making a diagnosis with the help of relevant investigations, defining affliction and extent of damage to organ(s) or body parts and determining drugs, its volume and duration of treatment to affect a cure is an art. The intervention and anticipating the treatment response is described as "artistic application of science". The diagnosis and treatment executed based on more and more evidence generated from research is important to predictably cure the ailment.

The modern (allopathy) medicine was started in Europe, Ayurveda in the Indian subcontinent and Chinese medicine at about the same time (3000 years). All three were based on religious and philosophical assumptions and thus the treatment executed was empiric. The practical medical experiences by the clinicians were passed down orally through numerous generations. "Sushruta Samhita" and Charak Samhita were written around 6th century BC. Sushruta Samhita detailed surgical techniques of making incisions, probing, foreign body extractions, alkali and thermal cauterizations, tooth extractions, caesarean section, and other surgical procedures (1). Thus, Ayurveda was far ahead of Western medicine in surgery.

Modern medicine also had a long history of pre-scientific stage of development. The clinicians practicing modern medicine applied proper scientific principles over the years and discarded old superstitious ideas with no genuine medical foundation. The observations were recorded and published in scientific journals, thus modern medicine evolved and developed to what it is today. Ayurveda did not evolve scientifically, hence continued in pre-scientific stage. Currently word medicine is synonymous with modern medicine and Ayurveda and Chinese medicine are called alternative medicine.

Whenever a clinical problem is confronted by a clinician, he/she tries to identify the cause and evolve treatment strategy to treat it. The outcome of treatment when scrutinized and assessed on scientific parameters, becomes an evidence. The evidence generated on a series of similar patients (inclusion criteria's), evaluated

on scientific parameters becomes the definite evidence and a research. When the generated evidence is published in peer-reviewed journals, it can be retrieved beyond human life and is available worldwide for posterity. It also becomes the foundation stone for future research and guiding principle for current treatment remedies.

Spinal Tuberculosis

Tuberculosis (TB) is as old as human being on mother earth and bacteria and human being have lived in symbiosis (2). The mycobacterium has been demonstrated by molecular methods in Egyptian mummies and are found in remains of iron age. The treatment of TB has evolved as the medical science evolved. The discovery of organism and antibiotic against mycobacterium has improved the outcome of treatment. Patients with TB spine in pre-antibiotic era were treated in sanatorium (by high protein diet, rest, fresh air, and sunshine) with the hope for natural quiescence of disease. Only few patients used to survive and remained crippled and rest used to die. The introduction of antitubercular drugs allowed the TB lesions to heal, thus the objective of treatment now changed to achieveing healing with residual sequlae of spinal deformity and neural complications. The development of diagnostics, imaging, surgical interventions, and instrumentations, anesthetic care, intensive care has improved and the objective of treatment to "healing of the lesion with near normal spine" (2).

Treatment Practices-TB Spine (1988)

The patients of TB spine without neural complications were treated by ambulant chemotherapy and by middle path regimen (surgery when indicated) with neural complications, on the contrary western centers were following universal surgical extirpation. Since the diagnosis was based on plain X-rays, hence the patients were reporting for the first time with kyphotic deformity. The healing of TB spine with residual kyphosis and/or sequalae of neural deficit was the expected outcome. There was a controversy on the optimum duration of antitubercular treatment (ATT) as well as the need/ rational of surgical intervention and instrumented stabilization. The kyphotic deformity was an unsolved issue in planning the management.

Beginning of the Journey of TB Spine Research

We encountered a case (1988) of late onset paraplegia (grade III) 20 years after initially being treated by anterolateral decompression (TB spine D3-4 with paraplegia). He had no symptoms of active disease of TB spine at this presentation. While analyzing the cause of late onset paraplegia, magnetic resonance imaging (MRI) scan, a new imaging modality at that time, available in an army establishment was performed. The spinal cord showed severe cord atrophy, and syringomyelia with healed vertebral lesion, severe kyphotic deformity and cord impingement by internal salient. Two other similar patients of late onset paraplegia also showed severe cord atrophy. Hence, we reported that severe cord atrophy and syringomyelia are attributed as the causes of late onset of paraplegia (3). These 3 cases stimulated us to explore MRI observations in TB spine with or without neural deficit: a) to diagnose spinal TB by MRI well before a deformity develops; b) diagnostic features of TB spine and features of healed disease by MRI; and c) defining MRI correlates of recovering/ non-recovering tuberculous paraplegia.

MRI Diagnosis of TB Spine

Forty-nine consecutive patients diagnosed clinico-radiologically and/or histology/fine needle aspiration cytology/AFB smear/molecular method (PCR) were enrolled. The MRI findings in these patients were analysed for diagnostic criteria for TB spine and

healing changes (4). The tubercular lesions were more extensive on MRI than that appreciated on X-rays as mean vertebral body (VB) affection on X-rays was 2.61 (n=128 VB) and on MRI 3.2 (n=161 VB). On MRI contiguous VB involvement, relative preservation of disc space, subligamentous paravertebral collection (low signal in T1WI and bright on T2WI), septate paravertebral collection, marrow edema, and epidural involvement (canal encroachment), intraosseous abscess, were considered diagnostic features of spinal TB. The patient not showing classical findings on MRI, but are suspected tubercular lesion (case of diagnostic dilemma) must be subjected for tissue diagnosis before starting ATT. The resolution of marrow edema and collection, fatty replacement of bone marrow, and resolution of cord signal intensity and absence of contrast enhancement were observed with healing/healed disease.

Early Diagnosis of Spinal TB before Development of Deformity

Spinal TB is a slowly developing disease. The bony lesion can only be appreciated if bone losses 40% of its calcium content (5). Thus radiological findings appear almost 3-4 months after the onset of disease. The patient continue to remain symptomatic during these 3-4 months. Since MRI could depict inflammation earlier, hence can be useful to diagnose TB spine in predestructive stage of disease. We defined the clinical or clinico-radiological predictors to suspect spinal TB lesion in early stage of disease. The patient with persistent localized back pain (3 months or more) with or without tenderness or spasm, with or without constitutional symptoms, with or without early radiological finding should be labeled as "observational spine" in the endemic zone for TB and need to be serially evaluated by X-rays. With subtle X-rays findings, the patient should be subjected to MRI. These X-ray findings of early suspicion will be variable depending on segment of spine affected (6, 7).

Cervical spine

The prevertebral soft tissue space (PVSTS) in cervical spine contains loose areolar tissue where inflammatory exudates collects when the underlying VB is affected by TB and can be appreciated as increase in PVSTS on X-rays The normal PVSTS space is described in millimeters, but it may be variable depending on height and built of the patient and particularly difficult to measure in digital X-rays. The normal PVSTS on lateral radiograph of the cervical spine (n=300) was calculated and described in relation to the width of the underlying VB. Before the bifurcation of nasopharynx into esophagus and trachea, PVSTS is one third and after the bifurcation it is two third of the width of VB behind. In front of C7, it is always less than C6. In front of the upper dorsal spine it should be 8–10 mm away from the vertebra and should follow the contour of upper dorsal spine, i.e., concave interiorly (8). Any localized or generalized increase in PVSTS is an indirect indicator of pathology in underlying bones. On observing increase in PVSTS the underlying bone disease may be diagnosed on MRI. Below D4–D5 the intervertebral disc height can be very well-appreciated on plain X-rays. The disc height on plain X-ray at any level should be equal or more than the disc above except D12–L1 and L5–S1. The early reduction of disc height in a symptomatic patient can be considered a suspected case of TB spine and with MRI can be diagnosed in inflammatory stage of disease well before a spinal deformity develops. If the treatment is started in predestructive stage (inflammatory stage), it would heal with almost near normal spine.

Tuberculous Myelopathy (Correlation of Clinical Course and MRI Observations)

Sixty patients of TB spine with para-quadriplegia were serially evaluated by MRI, while on treatment and 143 MRI scans were analysed (9). The cord compression was of two types: a) Liquid compression, having low signal

intensity on T1WI and bright signal on T2WI; b) Dry compression diagnosed as mixed extradural collection with the heterogeneous signal in T1WI and T2WI. The changes observed on spinal cord were: (i) cord edema observed as diffuse hyperintensity in T2WI and hypointensity on T1WI; (ii) myelomalacia as patchy hyperintensity in T2WI and hypointensity in T1WI; (iii) cord atrophy observed as a reduction in cord volume; and (iv) syringomyelia as dilatation of the central canal.

The predominant liquid compression, preserved spinal cord volume, and cord edema was observed in all those patients who recovered neurologically after treatment (non-operative or surgical) provided the patient does not show any imaging features of instability (like panvertebral disease).

The patients showing mixed extradural compression (dry lesion) and thick dura-arachnoid complex with diseased tissue encircling the spinal cord with features of myelomalacia are unlikely to show neural recovery and are indication for early surgical decompression.

Canal Encroachment

Intraspinal encroachment by granulation tissue is also observed in MRI scan even when patient has no neural deficit. MRI/CT scan of 16 cases of TB spine with no neural complications were enrolled (10). The area of the spinal cord and the spinal canal were calculated at the level of maximum compression in axial view. Upto 76% canal encroachment was observed in these patients and they did not have imaging features of instability (pathological subluxation/dislocation). The cord compression in the TB spine is gradual and spinal cord has time to adapt to gradually building cord compression. The neural deficit may develop at lesser canal compromised in the presence of spinal instability.

Spinal Deformity

In lesser resource countries the patients of TB spine generally present for the first time with spinal (kyphotic) deformity. The spinal deformity either remains static or increase while on treatment. If the kyphotic deformity heals with 60 degrees or more, the patient may present later with late onset paraplegia (paraplegia with healed disease). The surgical correction of healed kyphotic deformities is fraught with complications, hence all those patients who presented with 60 degree kyphotic deformity or likely to heal with 60 degree or more deformity should be surgically corrected in active disease. The final kyphosis can be predicted in adults before the start of treatment in active disease. Rajasekran *et al* proposed a formula to predict final kyphosis (11). We analysed 70 patients who were treated non-operatively (n=40) and by uninstrumented surgical decompression (n=30) (12). The final kyphosis recorded was correlated with initial VB height loss. The prediction of final kyphosis was better in non-operative group. The final kyphosis of 66.5 degrees +/-10 degree will occur in a patient with initial VB loss (IVBL) of more than two VB height. The patient with IVBL of 1.5 VB height or more in dorsal and dorsolumbar spine should be surgically treated by deformity correction.

Surgery in Spinal TB

The surgical decompression in patients of spinal TB with paraplegia having paretic intercostals with/without concomitant pulmonary disease is risky and requires excellent spinal surgery infrastructure. In underdeveloped countries, the infrastructure for complicated spinal surgery is sparse and disease burden is always more than available infrastructure. The Randomized controlled trial was performed where surgical decompression surgery in the dorsal spine by the transthoracic transpleural approach and extrapleural anterolateral approach were evaluated for neural

recovery and adequacy of surgical decompression. Both the procedures showed adequate and comparable quality of surgical decompression. Besides anterior decompression concomittent posterior instrumentation can also be performed by the extrapleural anterolateral approach. "T" incision was advocated instead of semicircular incision to increase the extent of exposure for instrumented stabilization (13-15). Hence, complex transthoracic anterior decompression is now rarely advocated as surgical options in TB spine of dorsal and dorsolumbar spine.

Need of Instrumented Stabilization in TB Spine

Lots of articles were published using instrumented stabilization. All authors though have used instrumented stabilization but have not described any indication for instrumented stabilization and it was performed with the objective to prevent on treatment deterioration of the kyphotic deformity. A total of 124 published articles from 1986 to 2006 where instrumentations have been used were analysed and published (16). None of the studies have defined indications to instrumentation and the rationale for the use of type of implant. Most of the authors have performed instrumented stabilization of the spine to prevent the postsurgical deterioration of the kyphosis (16, 17). The indications of instrumented stabilizations were listed by us as: (i) grossly unstable or potentially unstable spine (panvertebral disease) to prevent pathological subluxation/dislocation and the consequent development of severe neural deficit; (ii) long segment disease- the posterior instrumentation should be performed in cases with 4 or more vertebral body affection and, junctional region TB to support anterior bone graft to bridge the gap created after decompression; and (iii) instrumentation is an essential step as a part of procedure of surgical correction of kyphotic deformity.

Surgical Correction of Kyphotic Deformity

Since the surgical correction of kyphotic deformity is a complex surgery, hence each case has to be planned for the surgical approach and steps of deformity correction. A series of patients with TB spine who presented with kyphotic deformity were treated by deformity correction by the extrapleural anterolateral approach. The issues in kyphotic deformity correction surgery of spinal TB need to be addressed are (a) retropulsed tissue in the spinal canal directly compressing the spinal cord; (b) shortened vertebral column of longstanding and spinal cord has adjusted to long standing shortened length of vertebral column. Both issues can be addressed by anterior corpectomy, and posterior column shortening. The steps for deformity correction are anterior corpectomy, posterior column shortening, instrumented posterior stabilization, and anterior and posterior bone grafting (both) performed simultaneously and sequentially. The extrapleural anterolateral approach allows all steps to be performed simultaneously with posterior Hartshill instrumentation in dorsal spine. Since the kyphotic deformity is common in dorsolumbar spine, hence, a series of patients were taken for the same procedure performed by extrapleural retroperitoneal approach for thoraco-lumbar lesions. These two approaches allow surgical correction of kyphotic deformities in less resource infrastructures (18, 19).

Optimum Duration of Antitubercular Therapy (ATT)

Multiple studies have provided some evidence on duration of ATT which is variable between 6/9/12/18 months. This controversy can only be resolved if the end-point of treatment is defined. Well defined end-point of treatment does not exist as most of the articles have considered clinical/clinicoradiological, and hematological criteria with no recurrence for 2 years as healed status. MRI based criteria to

define healed status were taken by us to stop ATT in a prospective study to establish the efficacy of extended DOTS Category I of chemotherapy in spinal TB (20). Fifty two patients were evaluated by contrast MRI while on ATT; 35.2% patients attained healed status at 8 months, 60% at 12 months and 90% at 18 months. It was concluded and considered unscientific to stop ATT by fixed time schedule and we need to evaluate all spinal tubercular lesions at 8 months and subsequently to observe MRI signs of healed status before deciding stoppage of ATT. Since these patients were followed-up on contrast MRI which shows inflammation early and inflammation on MRI does not differentiate on active disease or healing disease, hence we evaluated a series of 30 patients by contrast MRI and PET scan. In an unpublished report we concluded that PET scan shows complete absence of the fluorodeoxyglucose (FDG) uptake when the TB spine lesion heals, even in the presence of contrast enhancement of the lesion on MRI. The duration of ATT to achieve healing of the lesion was found to be variable from 12 months to 24 months. This prospective study reiterated the observation of previous study that TB spine patient should be evaluated at 9, 12, 18 months and lesion should not be left until it attains the healed status.

Failure of Conservative Treatment

The failure of response to ATT could be due to paradoxical reaction or drug resistance of *Mycobacterium tuberculosis.* We evaluated prospectively a series of therapeutically refractory cases of TB spine (21). All patients who did not show clinical improvement on ATT or the lesion or spinal deformity deteriorated while on ATT or a new lesion appeared or wound dehiscence occurred at 5 months of ATT were included. These patients were labeled as presumptive drug resistance. The lesions were debrided and tissue was submitted for BACTEC culture. Two of 14 cases only demonstrated drug resistance bacteriologically, although histological diagnosis was TB in all. The remaining patients were treated as cases of clinical drug resistance on the protocol of multi-drug resistant (MDR)-TB. All patients showed healing of lesion on contrast MRI/PET scan. Since we had a poor percentage of proving drug resistance we continued to analyze another series of cases where the tissue after debridement was also submitted for Cartridge-based Nucleic Acid Amplification Technique (CB-NATT), Line Probe Assay (LPA) and Liquid Culture also. The molecular method and liquid culture improved the diagnosis of drug resistance.

Atypical Presentation of Spinal TB

Atypical spinal TB including intraspinal granuloma present with unique diagnostic challenges, hence are likely to be a missed diagnosis in initial stages of disease and later diagnosis is made when they develop complications. The intraspinal tubercular granuloma is one third of all spinal tumor syndromes in endemic region for TB. Extradural granuloma show good neural outcome after surgical decompression and peeling of granulomas, while intra-medullary granuloma respond/resolve with supervised ATT (22, 23).

While working and analyzing TB spine during the last 28 years, we reported pseudoaneurysm of aorta (24) and written review articles highlighting the consensus achieved and listing unanswered questions in TB spine of adults and children (25-27). We received 1470 citations (source Google Scholar) on the published articles. I was also invited to be the Guest Editor for a Symposium on "Management of Spinal TB" in "Clinical Orthopedics and Related Research" which got published in July (Vol. 460), 2007 Issue (28). Later on Indian Journal of Orthopaedics also

published a Symposium Proceedings in 2012 (29). In the recent meta-analysis performed by Wang et al (2017) on "Trends of Spinal Tuberculosis Research (1994-2015)- A Bibliometric Report", it was stated that "Dr Jain AK has published most papers in this field (n=20)" (30). All the discussed research and observations lead to resolution of a number of issues by consensus on:

a) Clinicoradiological predictors to suspect and diagnose spinal TB early in the pre-destructive stage before a complication develops,
b) The diagnostic features of TB spine on MRI,
c) MRI correlation for recoverable/non-recoverable paraplegia,
d) Define preference for surgical approaches for TB spine,
e) Indications of instrumented stabilization in TB spine,
f) Prediction of final kyphosis and correction of kyphotic deformity,
g) Treatment protocol of suspected drug resistance, and
h) Optimum duration of ATT is still under study, although 2 studies have concluded that TB spine should not be treated by fixed time frame and the lesion should be evaluated by contrast MRI and PET scan to demonstrate attainment of healed state. Currently, we are working on the role of CB-NATT and Line Probe Assay in diagnosis of spinal TB.

Working for 28 years over 3300 patients, 44 publications, and 25 research questions could contribute significantly in solving unresolved gaps in the knowledge on spinal TB. Recently, framed guidelines for treatment of bone and joint TB as a team lead in collaboration with the Ministry of Health and Family Welfare in association with WHO (31) and published a book on: "Tuberculosis of Bones, Joints and Spine- Evidence Based Management Guide" (32), we could give evidence on most of the issues where a very low evidence was available and highlighted the areas where research is still to be performed to meet the unmet needs of patients living with bone TB (32).

Growth of Science

To succeed as a competitive marathon runner, we have to practice over many months/years to improve cardiac fitness, musculoskel *et al* resilience, ability to run small distance, middle distance and long distance and then the practice to improve timing. The bigger goal is broken into small practice sessions and finally attaining fitness for competition. The run has to start with single step to complete 42 kilometers. At no point the focus of athelete should break. The same is true with growth of science. If the thought process of a researcher wavers in heterogeneous directions than effective output of the clinical solutions is very low even if a clinician is working overtime to solve multiple research questions. Although lot of research papers are published by one researcher, it still does not bring a perceptible change in clinical practice. One research question may answer a query. To change a practice one requires a series of research questions/clinical situations needing specific answers. If a clinician works slow and steady by a series of sequential research questions and by sustained and focused efforts, the research output can change the clinical practice. Mark Twain once said "The secret of getting task ahead is getting started." Break your complex overwhelming tasks into smaller manageable tasks than start working on one by one. While moving in one research direction many more new dimensions will be perceived. Success is the sum of small efforts made to answer research questions day in and day out.

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Therapies for Glomerular Diseases in Children

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ABSTRACT

Nephrotic syndrome is an important chronic disease of childhood, with a steroid sensitive course in most patients. Research on pathogenesis has emphasized the importance of T-lymphocyte dysregulation and vascular permeability factors that alter podocyte function and glomerular permselectivity. Mutations in genes that encode important podocyte proteins and therapeutic targets within podocytes have been identified. A hypothesis unifying available evidence on pathogenesis is yet to be proposed. An important proportion of patients have difficult disease course, characterized by frequent relapses, steroid dependence or steroid resistance, requiring therapy with alternative immunosuppressive agents. Clinical studies support the use of levamisole, cyclophosphamide, mycophenolate mofetil, calcineurin inhibitors (CNIs) and rituximab in patients with frequent relapses or steroid dependence. The management of steroid-resistant nephrotic syndrome is difficult and patients failing to achieve remission show progressive renal damage. Prospective studies in patients with steroid sensitive and steroid resistant nephrotic syndrome are the basis of current guidelines while ongoing studies will help identify and formulate effective and safe therapies.

Keywords: Calcineurin inhibitors, focal segmental glomerulosclerosis, minimal change disease, Rituximab.

Introduction

Glomerular diseases constitute a significant proportion of kidney diseases in children. They are responsible for a variety of clinical presentations that range from isolated hematuria and/or proteinuria, hypertension, acute nephritic or nephrotic syndrome, to acute kidney injury and chronic kidney disease of variable severity. Nephrotic syndrome is one of the most common chronic disorders of childhood with significant risk of acute and long-term morbidity. However, its pathogenesis remains unclear and therapies are largely empirical. This review focuses on the current understanding with respect to the pathogenesis and management of idiopathic nephrotic syndrome.

Nephrotic Syndrome

Nephrotic syndrome is characterized by massive proteinuria, hypoalbuminemia (albumin <2.5 g/dL), hyperlipidemia and edema. Data from single, multicenter or nationwide studies show that the incidence of nephrotic syndrome varies from 2-7 and prevalence 14-16 per 100000 children (1, 2). More than 90% are primary (idiopathic); a secondary cause is rare. Most (~80%) children with the idiopathic form of illness show remission following therapy with oral steroids. The prognosis in these cases is

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favorable, in contrast to patients who are steroid resistant.

Pathology

Histological studies by the International Society for Kidney Disease in Children (ISKDC) and our center show that almost three-quarter patients have insignificant glomerular changes on light microscopy (minimal change disease) (3). While immunofluorescence examination is usually normal, ultrastructure reveals effacement of podocytes. About 40-70% patients with steroid resistant and 5-10% cases with sensitive nephrotic syndrome have focal segmental glomerulosclerosis (FSGS). FSGS is classified, into five morphologic variants, based on location of sclerosis: tip lesions, cellular variant, perihilar lesions, collapsing FSGS and FSGS not otherwise specified (4). Collapsing glomerulopathy is associated with HIV, heroin intake and parvovirus infection. About 10-15% of patients with steroid resistance show features of C3 glomerulopathy, membranous nephropathy or IgA nephropathy.

Pathogenesis

The filtration barrier comprises of capillary endothelial fenestrations, glomerular basement membrane (GBM) and interdigitating podocyte processes. Studies show that podocytes are critical in maintaining selective filtering function. Application of high-throughput next-generation sequencing shows defects in genes encoding key proteins of podocytes in 80-100% cases with congenital nephrotic syndrome (onset <3 months age), and 50-60% of infantile-onset, 65-70% of familial and 25% of sporadic steroid resistant disease (2, 5, 6). Mutations in many genes are recognized: those encoding structural elements of slit diaphragm or podocyte cytoskeleton (*NPHS1, NPHS2, CD2AP, TRCP6, ACTN4, MYO1E*), proteins in the GBM (*LAMB2*), mitochondrial genes (*COQ2*), transcription factors (*WT1, LMX1B*), cubilin (*CUBN*), rhoGDIα (*ARHGDIA*) and inverted formin 2 (*INF2*). Although most patients with inherited forms of steroid resistance do not respond to immunosuppressive agents, partial response to calcineurin inhibitors (CNIs) is reported. Disease due to genetic defects is likely to progress to end-stage kidney disease and unlikely to recur in the allograft (2, 7, 8).

There is evidence of immune dysfunction in steroid sensitive disease. Altered cell mediated immunity and T-helper type 2 (Th2) polarization is proposed to, through undefined mechanisms, result in increased glomerular permeability. Recent studies suggest that the steroid sensitive illness is associated with an imbalance between Th 17 cells and regulatory T (Treg)-cells (9, 10). Deficiency or dysfunction of Treg cells may allow activation of effector T-cells to secrete factors that mediate glomerular permeability or increase oxidant production (11). Conversely, stimulation of Treg cells following measles or B-cell depletion with rituximab, induce sustained remission in minimal change disease (12, 13). Recent studies suggest that increased podocyte expression of CD80, soluble angiopoietin-like 4 (ANGPTL4) and microRNA might have a role in pathogenesis of proteinuria (14-17).

Finally, podocytes are recognized as a target for antiproteinuric interventions. Incubation of podocytes with corticosteroids, CNIs and rituximab has been shown to stabilize the actin cytoskeleton and restore distribution of key podocyte proteins, ameliorating proteinuria.

Circulating Factors

The soluble mediator hypothesis, supported by recurrence of nephrotic range proteinuria following transplant in 20-40% patients with idiopathic FSGS, induction of proteinuria and podocyte effacement in rats, or increase in vascular permeability in guinea pigs by supernatants from T-cells, is an accepted paradigm for disease pathogenesis. A number of

circulating factors have been proposed, including soluble urokinase plasminogen activating receptor (suPAR), interleukin (IL)-13, cardiotrophin like cytokine-1, tumor necrosis factor-α hemopexin and c-Maf inducing protein (18, 21).

Evaluation

Most patients with idiopathic nephrotic syndrome have steroid sensitive illness. The course varies with 35-40% having a single episode or 1-2 relapses and 55-60% showing multiple relapses that occur infrequently or frequently. Investigations at the onset include: (i) urinalysis; (ii) blood levels of urea, creatinine, albumin, cholesterol; and (iii) complete blood counts. Additional investigations, apart from a tuberculin test and chest X-ray, are rarely required. Most patients do not require a kidney biopsy. A biopsy is required at onset if a cause other than minimal change disease is suspected, such as: (i) age at onset <1 year or >16 year; (ii) gross or persistent microscopic hematuria, or low C3; (iii) renal failure not attributable to hypovolemia; (iv) suspected secondary cause; and (v) sustained severe hypertension. A renal biopsy is considered later for steroid resistance or if therapy with CNIs is planned (2, 22-24).

Steroid resistance is diagnosed if patients continue to show non-response (3-4+ proteinuria, edema or hypoalbuminemia) despite therapy with prednisolone in adequate doses for 4-8 weeks (23, 24). Recent recommendations suggest awaiting remission for 6-8 weeks while tapering corticosteroids; the use of pulse steroids to confirm resistance is not recommended. Patients with steroid resistant nephrotic syndrome require: (i) 24-hour quantitation of proteinuria; (ii) estimation of glomerular filtration rate; and (iii) renal biopsy. Testing for mutations are currently not recommended due to variable availability and high cost of testing and unclear association with response to therapy (2, 5, 24). Screening for genetic mutations is recommended for patients with family history of similar renal disease, those presenting in the first 3-6 months of life and those not responding to therapy with steroids and CNIs.

Steroid Sensitive Nephrotic Syndrome (SSNS)

Collaborative international efforts of ISKDC and Arbeitsgemeinschaft für Pädiatrische Nephrologie (APN) (25, 26) have helped refine the treatment of nephrotic syndrome. A number of expert groups have proposed guidelines for the diagnosis and management of patients with SSNS (Table 1) (22, 27, 28).

Initial Episode

Although the ISKDC proposed that the initial prednisolone therapy comprise of 4 weeks daily and 4 weeks intermittent treatment (25), there was evidence that prolongation of therapy to 12 weeks was better in terms of reducing the risk of frequent Relapses (26). Few experts suggested that extending therapy to 24 weeks was even better. Four recently published well designed RCTs that enrolled almost 800 patients emphasize that prolonged initial therapy for 4-6 months is not useful in modifying the course of the disease, or reducing subsequent need for steroids and other agents (29-31). Given this data and risk of corticosteroid adverse effects, we do not recommend prolongation of initial therapy beyond 12 weeks.

Frequent relapses

Risk factors for the occurrence of frequent relapses or steroid dependence include an early age of onset (<3 years), delayed time to initial remission, and brief initial corticosteroid therapy (2, 31). Patients with frequent relapses are at risk of corticosteroid toxicity as well as complications of nephrotic syndrome, including infections, thrombosis and dyslipidemia. Many patients require therapy with steroid sparing agents that maintain remission while limiting

exposure to corticosteroids; agents used are listed in Fig. 1 and Table 1. Medications that have been used for this purpose include long-term prednisolone, levamisole, cyclophosphamide, mycophenolate mofetil, cyclosporine, tacrolimus and rituximab (32-37). Since few RCTs have compared the relative efficacy of these medications, most guidelines do not specify the order or choice of therapy (22, 27, 28).

Table 1: Guidelines for managing steroid sensitive nephrotic syndrome

	Indian Society of Pediatric Nephrology (ISPN) 2008 (22)	**Kidney Disease: Improving Global Outcomes (KDIGO) 2012(27)**
Initial episode	Prednisolone 2 mg/kg (max. 60 mg) daily for 6 wk 1.5 mg/kg (max. 40 mg) alternate day (AD) for 6 weeks; discontinued without taper	Predniso(lo)ne 60 mg/m^2 daily for 4-6 weeks 40 mg/m^2 AD for 2-5 months, taper Total duration: ≥12 weeks
Relapse; infrequent relapses	Prednisolone 2 mg/kg daily until remission[#] 1.5 mg/kg AD for 4 weeks; discontinued	Prednisolone 60 mg/m^2 daily till remission[#] 40 mg/m^2 AD for ≥4 weeks
Frequent relapses, steroid dependence	Long term AD prednisolone: 0.5-0.7 mg/kg for 9-18 months	Long term prednisolone: lowest dose AD for ≥3 months Administer daily during respiratory & other infections Consider low dose daily without major adverse effects, if AD therapy ineffective
	Corticosteroid sparing agents: Steroid threshold >0.5-0.7 mg/kg; steroid toxicity	*Corticosteroid sparing agents*: Use if steroid toxicity
	Levamisole: 2-2.5 mg/kg AD for 1-2 years	Levamisole: 2.5 mg/kg AD for ≥1 year
	Cyclophosphamide[$1]: 2 mg/kg daily for 12 weeks Chlorambucil: Not recommended	Alkylating agents: For frequent relapses, dependence; avoid second course; initiate therapy in remission
	Calcineurin inhibitors[$2]: Cyclosporine 4-5 mg/kg, tacrolimus 0.1-0.2 mg/kg daily for 1-2 years; monitor levels if toxicity, non-compliance, unsatisfactory response is suspected	Calcineurin inhibitors: Cyclosporine or tacrolimus for ≥1 year; use latter if unacceptable cosmetic side effects with cyclosporine; monitor levels during therapy
	Mycophenolate mofetil: 800-1200 mg/m^2 daily for 1-2 years	Mycophenolate mofetil: 1200 mg/m^2 daily for ≥1 year
	Mizoribine, azathioprine: Not mentioned	Mizoribine, azathioprine: Suggest that not be used
	Rituximab: Not mentioned	Rituximab: If failing other agents, serious adverse effects

[#]Urine protein trace or nil or urine protein to creatinine ratio <200 mg/g for 3 consecutive days;
[$1]Prefer CNIs if: significant steroid toxicity, severe relapses (with hypovolemia or thrombosis), poor compliance or difficult follow up;
[$2]Prefer CNIs if: continued dependence or frequent relapses despite treatment with agents listed previously.

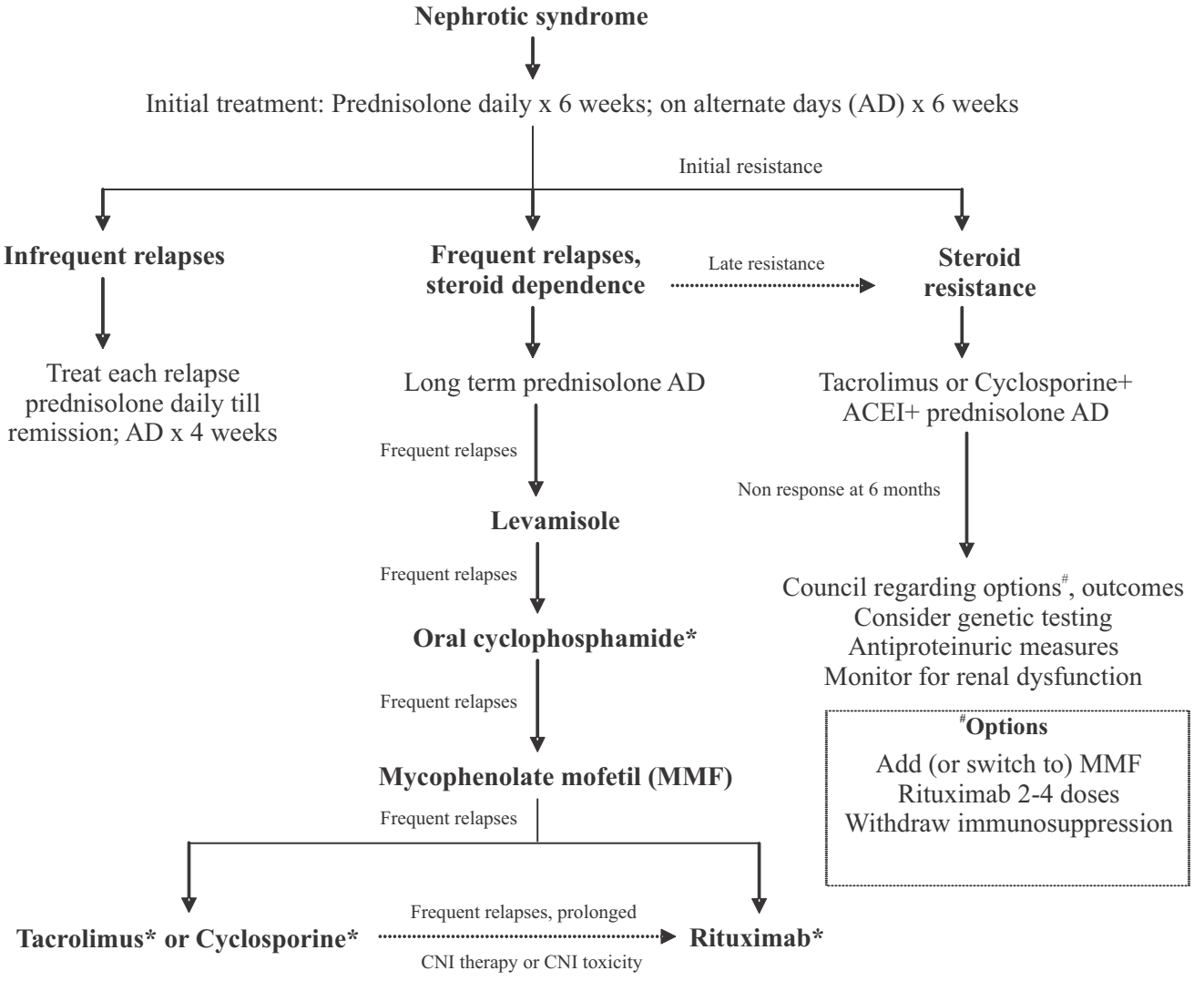

Fig. 1: Summary of therapy of steroid sensitive and steroid resistant nephrotic syndrome Medications are usually recommended in order from top to bottom. Agents marked with asterisk (*) are preferred in patients with significant steroid toxicity (cataract, severe stunting, obesity) or if relapses are associated with severe complications (thrombosis, severe infections). Patients with steroid resistance should receive treatment with a calcineurin inhibitor (CNI), an angiotensin converting enzyme inhibitor (ACEI) or angiotensin receptor blocker and tapering doses of prednisolone. Since response to other strategies is less satisfactory and non-response to CNI is associated with adverse outcomes, further immunosuppression should be considered following counseling regarding efficacy, safety and costs of various options.

Management of Steroid Resistant Nephrotic Syndrome

Therapy of patients with steroid resistant nephrotic syndrome is difficult, with variable response to immunosuppression, adverse effects of prolonged therapy and risk of progressive renal damage. Table 2 and Fig. 1 summarize guidelines on the evaluation, management and definitions of response (23, 24, 28). Most regimens combine daily therapy with a CNIs, angiotensin converting enzyme (ACE) inhibitors, and alternate day prednisolone (23, 24, 28, 38, 39). Cyclosporine A (CsA) and

Table 2: Guidelines for management of steroid resistant nephrotic syndrome

	Indian Society of Pediatric Nephrology, 2009 (23)	**Kidney Disease: Improving Global Outcomes (KDIGO), 2012 (24)**
Definition	Lack of remission[#] despite treatment with prednisolone at 2 mg/kg/day for 4 weeks; exclude systemic infections	Lack of remission[#] despite treatment with predniso(lo)ne for 8 weeks (2 mg/kg/day for 4 wk; 1.5 mg/kg on alternate days for 4 wk)
Evaluation	Kidney biopsy Serum creatinine, albumin Screen for mutations: Familial or congenital forms; patients with initial resistance	Kidney biopsy eGFR; 24-hr or spot protein, creatinine Screen for genetic mutations
Therapy	Choice based on preference and costs (i) Calcineurin inhibitor (CNI): cyclosporine or tacrolimus: efficacy 60-80% (ii) Cyclophosphamide: efficacy ~40% (I.V.), 25% (oral) (iii) I.V. methylprednisolone, oral cyclophosphamide: efficacy 30-50%	CNI with low dose prednisolone: Efficacy 69-86% Patients without remission to CNI at 6 months Mycophenolate mofetil efficacy ~33% High-dose corticosteroids efficacy ~47% Combination of agents: Do **not** use cyclophosphamide or rituximab
	Non immunosuppressive therapies ACE inhibitors or ARB Statins if dyslipidemia >6 months	Non immunosuppressive therapies ACE inhibitors, ARB: Use in all; proteinuria reduction 33-62%
Assess response at 6-months	Both complete and partial remission acceptable Complete remission: trace/negative protein; Up/Uc<0.2 mg/mg Partial remission: 1-2+ proteinuria; Up/Uc 0.2-2 Non-response: 3-4+ proteinuria; Up/Uc >2; blood albumin <2.5 g/dL	Both complete and partial remission acceptable Complete remission: proteinuria <0.3 g/24-hr; Up/Uc <300 mg/g Partial remission: proteinuria >0.3 g but <3.5 g/24-hr; decrease in proteinuria by ≥50%
Duration of therapy	Discontinue CNI if no remission at 6 months CNI for 2-3 year if complete/partial remission Longer if no nephrotoxicity on repeat biopsy	Discontinue CNI if no remission at 6 months Continue CNI for ≥ 12 months if complete/partial remission at 6 months
Monitoring	Trough cyclosporine 80-120 ng/ml; tacrolimus 5-8 ng/ml eGFR: Maintain±20% of baseline Rebiopsy: Therapy >2-3 yr; suspected toxicity	Relapse after achieving remission: Treat with oral corticosteroids Use previously successful medication Use alternative agent to minimize cumulative toxicity

[#]Urine protein trace or nil or urine protein to creatinine (Up/Uc) ratio <200 mg/g for 3 consecutive days.

ACE-angiotensin converting enzyme; ARB-angiotensin receptor blockers; CNI-calcineurin inhibitor; eGFR-estimated glomerular filtration rate; Up/Uc-spot urine protein to creatinine ratio.

tacrolimus appear to have similar efficacy and low rates of adverse effects (40). The aim of therapy is to induce and maintain remission of proteinuria, while avoiding medication related adverse effects. Patients are monitored closely until response to therapy is demonstrated, and then every 3-4 months (23). While complete remission is associated with high rates of renal

survival, even partial remission is associated with satisfactory outcomes, compared to those with non-response (41). Consensus is lacking on the optimal duration of treatment with CNIs. The agent is usually continued for 2-3 years, followed by one of the following: (i) tapering to the lowest effective dose, and continued for another 1-2 years; (ii) exclude nephrotoxicity on renal histology, and continue therapy; and (iii) switch treatment to a less toxic agent, e.g. mycophenolate or rituximab.

Given the overall limited efficacy in pediatric patients and risk of significant toxicity, KDIGO and Canadian guidelines suggest not using cyclophosphamide for patients with steroid resistance (24, 28). However, the relatively low cost of IV cyclophosphamide still allows it to be an option in resource limited settings (23). Despite initial interest (42), the efficacy of rituximab in inducing remission in patients with steroid and CNI-resistant nephrotic syndrome is limited (43). A RCT on 31 children with steroid and CNI-resistant nephrotic syndrome failed to show benefits of additional rituximab therapy in ameliorating proteinuria at 3 and 6 months (44). Our experience on 58 patients with steroid- and CNI-resistance confirms limited efficacy, with complete and partial remission in 12.1% and 17.2% patients, respectively (36). Similar to previous findings, response to rituximab was better in patients with prior response to a CNI and unsatisfactory in those with FSGS. Therapy with rituximab is likely to maintain remission, reduce relapses and enable withdrawal of steroids and CNIs.

Outcomes

Most patients with steroid sensitive nephrotic syndrome show satisfactory outcomes. Morbidity due to infections has declined with their prompt diagnosis and use of vaccines. Steroid toxicity remains a major concern in patients with frequent relapses or steroid dependence. Follow-up of the initial ISKDC cohort revealed that almost 80% patients were in sustained remission at 8 years from diagnosis (25); other series suggest that ~25% patients continue to relapse into adulthood (45). Ten-year follow-up of patients who received CsA for frequent relapses in a randomized study showed that 17.4% and 50% continued to suffer infrequent or frequent relapses, respectively, into adulthood (46).

Outcomes in patients with steroid resistance are less satisfactory. Patients with minimal change disease show better prognosis than those with FSGS. The chief factor predicting renal outcome is the response of proteinuria to therapy rather than histology. Renal survival varies from 72-94% at 5 years, with resistance to CNIs and presence of FSGS predicting adverse outcomes (47, 48).

Recurrence of FSGS after Transplantation

Almost 30% of patients with idiopathic FSGS undergoing transplantation develop allograft recurrence, with risk of delayed allograft function and loss (30-50% at 5-year) (49). Recurrence of proteinuria occurs within hours to days after the transplant, and is characterized by hypoalbuminemia and foot process effacement. Risk factors for recurrence include: (i) white ethnicity; (ii) early onset of disease (<15-year); (iii) late rather than initial resistance; (iv) non-genetic forms of disease; (v) progression to end-stage disease within 3-year from onset; and (vi) nephrectomy of native kidneys prior to transplant (49-51). Disease recurrence is attributed to circulating permeability factors, the precise nature of which is unknown.

Despite the risk of recurrence, live donors are preferred in view of better overall outcome. Pre-transplant plasmapheresis is used to decrease the risk of recurrence (52). Therapy for patients with recurrent FSGS include one or more of the following: (i) intensive and prolonged plasmapheresis (53); (ii) rituximab (375 mg/m^2/week for 2-4 weeks) (43); (iii)

immunosuppression, including high dose CsA, cyclophosphamide (2-2.5 mg/kg/day for 3 months) instead of Mycophenolate Mofetil; (iv) I.V. immunoglobulin (500 mg/kg/dose once a week); and (v) ACE inhibition. Results of treatment with I.V. abatacept are unsatisfactory. Patients with refractory illness might benefit by intensive lipid apheresis, using specially designed columns (54).

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HEV-related Liver Disease in India : Why is the Disease Stormy?

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ABSTRACT

Hepatitis E virus (HEV) is an important cause of epidemic and sporadic acute viral hepatitis (AVH) in many developing countries, including India. Hepatitis E, a positive-sense single-stranded RNA virus approximately 7.2 kb in length had been classified provisionally into the Caliciviridae family from 1988 to 1998 but HEV is currently placed in the genus Hepevirus and is the only member of the family Hepeviridae. Pregnant women with jaundice and AVH caused by HEV infection have worse fetal and obstetric outcome and higher maternal mortality compared to other types of viral hepatitis. Studies from various developing countries have shown that the incidence of HEV infection in pregnancy is high and a significant proportion of pregnant women can progress to fulminant hepatitis with a mortality rate varying from 30% to 100%. The incidence of hepatitis B virus (HBV) related acute liver failure is known widely in comparison to hepatitis C virus (HCV) infection in which acute liver failure (ALF) is rare. But the severe course of HEV infection causing ALF during pregnancy is unique to this virus with chronicity occurring in recipients of solid organ transplants.

Various factors have been suggested to be associated with the mortality rate of the HEV in pregnant women along with the abortion of the fetus. Steroid hormones play a significant role in the viral replication through their effects on viral regulatory elements. The NF-κB signaling pathway regulating at the transcriptional level through p50 subunits has been suggested to correlate with the severe liver damage, leading to multiple organ failure and the death of both the mother and the fetus. Pregnant women in Asia suffer from folate deficiency reducing the immunocompetence to greater risk of multiple viral infections and higher viral load. The viral load of HEV was found to be significantly higher ($P < 0.05$) in pregnant patients compared to the non-pregnant and the viral copies of HEV with fulminant hepatic failure (FHF) in pregnant women were comparatively higher when compared to the pregnant women with AVH, which may be related to the severity of the disease in these patients. Besides, reduced expression of progesterone and progesterone induced-blocking factor and the high viral load of HEV have been regarded as a cause of poor pregnancy outcome in hepatitis E infection. Vertical transmission of the HEV infection has been reported. There are published reports of abortion, death of the fetus in utero, premature delivery or death of the baby soon after birth in patients with icteric hepatitis or with ALF caused by HEV. However, studies in Europe and United States have shown the course of viral hepatitis during pregnancy resembling with the non-pregnant women. In contrast, various reports carried out in India, Iran, Africa, and Middle East have reported the incidence of ALF to be higher during pregnancy.

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Data on the viral load of HEV during pregnancy are limited. The study was designed to determine the viral load of HEV and its association with the disease severity in patients with ALF. A total HEV related 163 patients with ALF which included 105 pregnant, 46 non-pregnant women and girls, 12 men, and 730 patients with AVH which comprised of 220 pregnant women; 282 non-pregnant women and girls, and 228 men were included. Viral load was measured by real-time PCR. Comparison was made between the pregnant and non-pregnant women. HEV RNA was detectable in 265 patients (142 pregnant; 75 non-pregnant and 48 men) and 104 patients with ALF (64 pregnant, 34 non-pregnant and 6 men). The viral load of HEV in pregnant women with ALF and AVH was significantly higher 129,984.0±103,104.17 and 768.92±1,105.40 copies/ml, respectively compared to the non-pregnant women which was 189.2±225 and 12.73±7.8 copies/ml ($P < 0.0001$). The viral load of HEV was also significantly higher in the pregnant patients with ALF compared to the pregnant women with AVH and also men ($P < 0.0001$). High viral load of HEV during pregnancy could be one of the factors responsible for the severity of the infection during pregnancy.

Keywords: Immunocompetence, HEV during pregnancy, acute liver failure, acute viral hepatitis.

Introduction

Viral hepatitis, caused by various hepatotropic viruses A through E, constitutes a major healthcare burden in India (1). Several epidemics of viral hepatitis have been reported (2-8). Both hepatitis A virus (HAV) (9) and hepatitis E virus (HEV) are transmitted by fecal-oral route and highly endemic in India.

HEV has a positive-stranded, 7.5 kb, RNA genome with 3 open reading frames (ORFs) (10, 11). HEV has been accountable for most of the epidemics of viral hepatitis in India (2-8, 12-19). HEV infection is responsible for 30-70% of cases of acute sporadic hepatitis and is the leading cause of acute liver failure (ALF) in India (20-23). HEV infection is a common cause of super infection leading to acute on chronic liver failure in patients with chronic liver disease due to various etiologies (24, 25). It is transmitted majorly by fecal contamination of water and food (2-8, 26). Thus, it is important to understand the epidemiology of HEV infection and take preventive measures against the propagation of the virus.

The first extensively studied epidemic of HEV infection in Delhi affected 29300 people between December 1955 and January 1956 (1) and much of the epidemiological information on HEV had been collected from this epidemic. A number of epidemics reported subsequently had identical epidemiological features (2-8, 12, 14). In most of the epidemics, fecal contamination of the source of drinking water was the culprit.

During epidemics of HEV infection, secondary attack rates in household contacts are 0.7-2% (10, 18, 27-29). Person-to-person transmission is uncommon in the course of sporadic HEV infection (28). However, a few studies have reported the possibility of parenteral transmission (28-35).

The incubation period of HEV infection is 2–9 weeks (mean 6 weeks) (2-8). In epidemics of HEV infection, clinical hepatitis occurs more frequently in adults than children below 15 years of age, and in men than women (2-8). Anicteric hepatitis is more common than icteric hepatitis during epidemics (2-5). Icteric sporadic hepatitis has been reported in children (28, 35).

In India, HEV infection has also been associated with severe liver disease. During epidemics, pregnant women (second and third trimester) are infected more frequently (12-20%) than men and non-pregnant women (2-4%) (2-8, 18, 19, 22). The incidence of ALF is

higher among pregnant women (10-22%) with HEV infection than among men and non-pregnant women (1-2%) (2-8, 18, 19). Therefore, mortality is considerably higher among pregnant women (10-39%) than in the general population (0.06-12%) who develop acute hepatitis during epidemics (2-8, 18, 19). HEV infection has been detected in 30-45% of patients with ALF in sporadic cases (22). Combined HAV and HEV infection is associated with ALF in children (36). Superinfection with HEV has been found to cause decompensation of compensated liver disease (24, 25). However, no chronic sequelae have been reported after HEV infection (37).

Hepatitis and Pregnancy

Pregnancy appears to be a potential risk factor for viral replication and leads extreme low immune status of Indian/Asian pregnant women. Mortality rates among pregnant women, especially those infected in the 3rd trimester, have ranged between 5% and 25%, much higher that men and non-pregnant women (38). It has been reported that a significant proportion of pregnant women with acute viral hepatitis E (AVH-E) (up to 70%) progress to ALF with a short pre-encephalopathy period, rapid development of cerebral edema and high occurrence of disseminated intravascular coagulation (39).

Vertical transmission of HEV infection from mother to infant, although rare, has been reported. Babies born to HEV-RNA positive mother had evidence of hepatitis E infection (40-42). Fulminant HEV infection in pregnancy contributes to highest mortality rate of the fetus and mother. The fatality rate among pregnant women with ALF is reported to be high in India at 22.2%, with the maximum severity occurring during the 3rd trimester (44.4%) (38, 43, 44). Hepatitis E in pregnancy is also associated with high rates of spontaneous abortion, intrauterine death, and preterm labour (38). Worse maternal and fetal outcome of Hepatitis E compared to other types of viral hepatitis has been observed in pregnant women with HEV infection (45). Greater morbidity and mortality, particularly during epidemics of hepatitis, has been noted among pregnant females in developing countries.

Association of HEV and viral hepatitis with pregnancy has been reported earlier in many studies. Jaiswal and colleagues (46) and Borkakoti *et al* (47) from India and Aziz and associates (48) from Pakistan have reported that HEV is responsible for 58-62% of cases of AVH in pregnant women, respectively. Two studies from New Delhi (42, 43) reported slightly lower prevalence (45% and 37%, respectively), and a study of sporadic HEV infection in the context of multiple HEV epidemics in Kashmir reported a prevalence of 86% among pregnant patients with AVH (40). Patra and colleagues in a study on pregnant women with jaundice and AVH caused by HEV infection concluded that, they had a higher maternal mortality rate and worse obstetric and fetal outcomes than did pregnant women with jaundice and AVH caused by other types of viral hepatitis (45).

HEV infection during pregnancy leads to severe complications which may result in fetal and/or maternal mortality, abortion, premature delivery, or death of a live-born baby soon after birth depending on the severity of the infection which is stratified as AVH or ALF (most severe form of AVH). HEV infection is one of the predominant causes of pregnancy-related complications in the developing countries including India (38, 49).

HEV infection accounts for 50-70% of all patients with sporadic viral hepatitis in India (50). The reason for it may be that pregnancy is associated with high levels of steroid hormones. These steroid hormones may promote viral replication. It also has a direct inhibition on hepatic cells, which may predispose to hepatic

dysfunction/failure when exposed to infectious pathogens (51). Steroid hormones are immunosuppressive (52) and mediate lymphocyte apoptosis through NF-κB. NF-κB is a eukaryotic dimeric transcription factor which has a multiple cellular effects, including liver development and regeneration and its implications on the immune response (53).

Jilani *et al* found that HEV infected pregnant women with fulminant hepatic failure (FHF) had lower CD4 count and higher CD8 counts, they also observed that the levels of estrogens, progesterone and beta-HCG were significantly higher in the above-mentioned group when compared to HEV negative patients or control healthy pregnant females (54). Although the levels of hormones were physiologically high in the normal control population; patients with HEV infection seemed to have significantly higher levels than controls, which probably explain the direct interaction of HEV with the immune system. In another interesting study, Pal *et al* studied the cellular immune response in both pregnant and non-pregnant women with acute hepatitis E and the control population (55); they found that pregnant women with HEV had generalized immune suppression characterized by decrease in lymphocyte response to phytohemagglutinin (PHA) with a predominant Th2 bias as compared to non-pregnant women with hepatitis E and normal healthy controls. This was contradictory to the earlier hypothesis that normal pregnancy is associated with systemic immune suppression with an increased risk of infections (56-59).

Higher viral load of HEV has been reported to be associated with FHF during pregnancy; this was reported in a study by Kar *et al*, where a comparatively higher HEV viral load was observed in FHF patients (139994.0±103104.17 copies/ml) than AVH patients (768.92±1105.40 copies/ml). However, HEV genotype could not be correlated with the disease outcome as only single genotype (genotype1) was detected in both the disease groups (60). High fetal mortality has been explained in AVH and FHF cases which showed vertical transmission of HEV from HEV infected mothers to their infants (41).

In a recent study by Deka *et al*, 2010 it was shown that PROGINS, i.e. anti-progesterone monoclonal antibodies carriers and lower expression of progesterone receptor (PR) and progesterone-induced blocking factor (PIBF), as well as high HEV load influences the Hepatitis E disease severity and outcome in pregnancy. Higher IL-12 to IL-10 ratio (Th1 bias) in FHF indicates, that after crossing the period when there was a lower IL-12 to IL-10 ratio and after the completion of HEV incubation period (i.e. 15-64 days), when the virus has started causing damage to the cells, cytotoxic immunity rises (Th1 immunological state) up to a particular level where body can fight against the virus infected cells but in the due process, lower PIBF expression and higher NK cell activity results in reduced fetal protection and eventually fetal death occurs because of immunological injury (61).

HEV Genotypes and Severity of Hepatitis E during Pregnancy

There are 4 mammalian genotypes of HEV found to have unique geographic distributions. Genotype 1 includes Asian and African HEV strains, genotype 2 includes the single Mexican HEV strain and few variants identified from industrialized countries and genotype 4 includes human and sine HEV strains from Asia, particularly China, Taiwan and Japan. HEV with genotype 1 is most frequently recovered from patients in developing countries (Asia, North Africa). This genotype and genotype 2 appear to be more virulent than genotypes 3 and 4 (62). It has been discussed earlier that the course and severity of hepatitis E in pregnant women is not different from that in non-pregnant women in Europe and United States. This can be explained by the viral genotypes with lesser virulence

found in those areas. In the United Kingdom, HEV genotype 3 is most common, like genotype 4 in China (63, 64).

When HEV infection occurs, a cytotoxic Th1 immune response is likely to be elicited in the Th2 biased pregnant women. FHF is always associated with high HEV load. For that a strong Th1 response is required. This elevated Th1 immune response if still remains insufficient to fight with such a high HEV load, there is a possibility that Th1 response goes on increasing but in the due process, the cytotoxic immune response may result in reduced fetal protection and eventually fetal death.

Opinions differ over the maternal and fetal outcome of pregnancies associated with viral hepatitis. The studies from West opinions differ over the maternal and fetal outcome of pregnancies associated with viral hepatitis. The studies from the developed countries conclude that the pregnancy state, per se, has no adverse effect on the course of hepatitis, provided the nutrition is adequate. However, increased maternal and fetal mortality has been reported by many groups, mainly from the developing countries. Poor prenatal care and maternal nutrition appear to have contributed significantly to the increased severity of infection.

HEV infection in pregnancy leads to poor maternal and fetal outcome. FHF patients show Th1 biasness in terms of higher IL-12/IL-10 ratio. Thus, this shift of Th2 biasness, which is a characteristic of normal pregnancy, in the HEV infected pregnant women, is suggestive of the role of immunological shift during hepatitis E-related FHF in pregnancy. This immune alteration in turn may lead to reduced fetal protection which is probably due to higher activity of NK cells leading to fetal death. Viral load is comparatively higher in FHF than AVH and also higher in patients with fetal mortality in both AVH and FHF, suggesting its role with the disease severity. High viral load and Th1 immunological state together may attribute to the poor pregnancy outcome in hepatitis E.

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Letter to the Editor

Tinea pseudoimbricata

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Sir,

Among all branches of Medicine, Dermatology is unique. Nowhere else does physical examination holds precedence over history. Professor Peter Mortimer, Professor of Dermatological Medicine at University of London, in his foreword to the first edition of Oxford Handbook of Medical Dermatology has astutely opined that, "Dermatology is arguably the most clinical of all Medical specialities because it relies less on investigation and more on good old fashioned observation and interpretation of symptoms and signs for diagnosis."

We therefore present an important facet of clinical observation. A teenage boy came to the Dermatology OPD with complaints of itching and raised, spreading lesions on the skin of chest and abdomen for the past two months. The problem began from the groin first, when he started applying creams that were kept in home. The itching diminished but the skin lesions started to develop at other spots on the trunk. We showed him samples of commonly abused combination creams, out of which he identified two. Both the preparations contained Clobetasol, which is a super potent steroid.

The examination of lesions revealed multiple annular plaques of various sizes, mostly seen over chest and abdomen with few plaques over back. The groin on both sides had similar plaques, which were showing tendency to spread downwards towards the thigh (Fig. 1). On close inspection in most plaques multiple 'rings within ring' were seen (Fig. 2). Also noted, was mild scaling and epidermal atrophy, the latter attributable to steroid abuse.

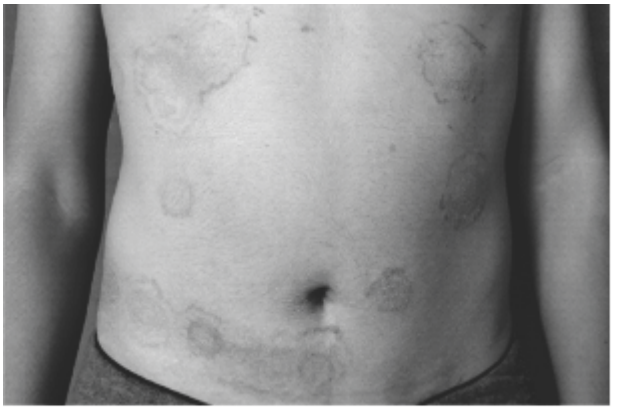

Fig. 1: Multiple, annular plaques of various sizes over chest and abdomen.

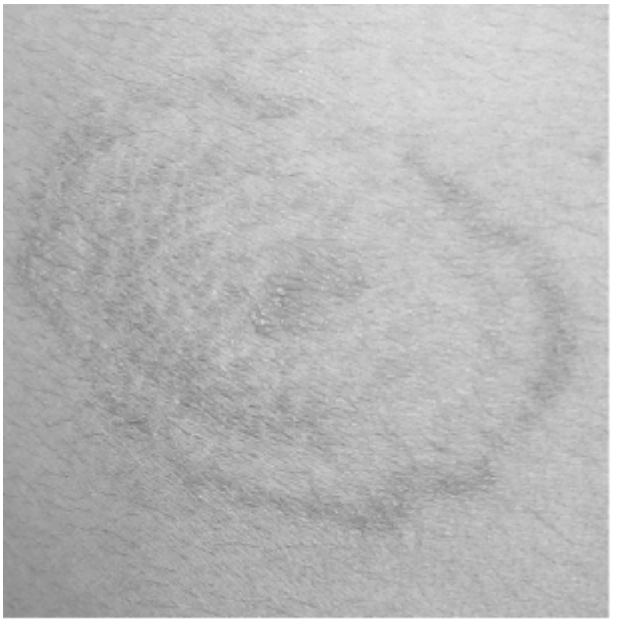

Fig. 2: Close up of 'Rings within Ring'

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A diagnosis of Tinea pseudoimbricata was made and the blood sugar of the boy was sent along with scraping for KOH mount. The blood sugar was within normal limits and the scraping showed multiple septate, hyaline hyphae arranged at acute angles.

It is noteworthy that, Tinea imbricata is a rare, superficial fungal infection caused by *Trichophyton concentricum*. It is endemic to the South Pacific and regions of Central and South America (1). It has many more concentric circles and is usually generalized. Tinea pseudoimbricata on the other hand refers to usually two or rarely, three concentric circles within a lesion of a 'Ring worm'. 'Rings within the ring' and 'double-edged tinea' are descriptions to familiarize non-dermatologists with its presentation. Its importance is that it should immediately raise the suspicion of topical corticosteroid abuse (2). Other causes of immunosuppression, which are clinically appropriate, should also be ruled out.

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