Editorial

PEDIATRIC HIV INFECTION: RECENT ADVANCES

Pediatric AIDS in many ways stands apart from other chronic life threatening childhood illnesses where only one family member is affected^ in AIDS, often the whole family is afflicted. The primary "care-provider" of the child, the mother, is the source of the child's illness, is almost always infected, and is often ill; the primary "bread-winner", the father, is most often the cause. The children of these parents, brought up in a situation of severe economic and social disruption, face extreme deprivation from all quarters. Pediatric AIDS should, therefore, be viewed in the broader perspective of the family and of the community at large, with a special focus on mothers.

Over the last few years, though a great deal has been learnt about this new illness, a large number of "grey areas" continue to exist. Newer data often contradicts earlier observations, and in view of these conflicting findings, controversies continue. The areas, that to my mind, have been subjected to the most intensive research are the factors responsible for mother to infant transmission, early diagnosis of HIV infection in infants and the therapy of pediatric AIDS.

Mother to Infant Transmission

More than 80% of cases of pediatric HIV infections occur through the vertical route and therefore any action targeted at reducing this rate of mother to infant spread, must focus on factors that influence this transmission. Though it is now clearly known that not all children born to infected mothers will be infected, the reasons for this are just being understood. In earlier studies, the rates of mother to infant transmission were estimated to be between 25-40%(l,2), but recent studies from the more developed countries indicate transmission rates between 13-15%(3,4). Whether these differences are because of selection bias is not clear because in the studies of Ryder *et* a/.(1) a much larger proportion of infected mothers had AIDS as compared to the investigations of the European Collaborative Study Groups(3,4). Moreover, the criteria for diagnosis of HIV infection in the babies was different in the two groups. Be as it may, there appears to be a difference in the rates of transmission between the developed and the less deve'oped countries, the rates being higher in the latter(5). As more information becomes available on maternal-fetal transmission and as techniques of early diagnosis improve, it is likely that wider variations in transmission rates will be found, depending on a multitude of epidemiologic factors including the stage of maternal disease and geographic location among others.

Though it is known that HIV can be transmitted during all stages of pregnancy and parturition, the relative proportions of infants infected *in utero,* during delivery or postnatally is not known

with certainty. HIV has been isolated from early aborted fetuses(6), giving irrefutable evidence of intrauterine transmission. However, in a number of cases, infection may not occur until delivery, at a time when the infant is exposed to large quantities of infected maternal blood and body fluids. It is now proved beyond doubt that HIV can be transmitted through breast milk(7), and it has recently been shown that "early" breast milk, which is more cellular, has a higher risk of transmitting infection(8). Though specific HIV-IgA (the usual secretory immunoglobulin) has not been detected in the milk of infected mothers, an unusual secretory IgM (IgM antibodies with a secretory component) has been found in some milks, and they are associated with a lower rate of transmission(8). Overall, breastfeeding by an infected mother adds an additional risk of around 14% over and above the usual risk of vertical transmission(7). "This is much higher than the earlier estimated risk when the mother to infant transmission by this method was thought to be occasional(9).

The factors that influence vertical transmission have come under scrutiny recently. Low CD4+ cell counts in the mother(l,4), high maternal viral load(10,ll), maternal p24 antigenemia(15) and low levels of anti-HIV antibodies(12) increase the risk of mother to infant transmission. The presence of high levels of anti-p24 antibodies and neutralizing antibodies do not influence the transmission rates(13). The greatest interest has, however, focussed around the role of specific maternal antibodies to some epitopes of the viral envelope glycoprotein, especially to the ones directed towards the principal neutralizing domain (PND) of the V_3 hypervariable loop of the gpl20 molecule. It has been shown that the presence of high affinity anti-gpl20 antibodies(13), high-affinity anti-PND antibodies(11) and anti-PND antibodies(14) are associated with lower rates of transmission.

Factors in the developing fetus and newborn that could contribute to vertical transmission include whether infection occured during gestation or at parturition, the time of infection relative to the infant's immune system, and whether the fetal or neonatal immune system was capable of responding to HIV. It is known that babies of HIV infected mothers can generate HlV-specific cytotoxic T-lymphocyte responses(15). Intrauterine priming to HIV *env* determinants can occur, and HIV-specific T-helper immunity can result from such exposure(T&). The fetal or neonatal immune system could, therefore, play an important role in the prevention of the establishment of infection in the newborn. The importance of these findings is that once the factors responsible for vertical transmission are known with certainty, and the methods to detect these factors are easily available, possible modes of prevention of vertical transmission may be possible.

Early Diagnosis of HIV Infection

In older children infected by other means, the diagnosis of HIV infection is fairly straight forward, and requires the demonstration of HIV antibodies. Because of its cost and convenience, the ELISA is the usual screening test. Despite their high sensitivity, earlier ELISA kits lacked specificity, especially

when used to screen low risk populations. The later generation ELISA kits use recombinant DNA proteins or synthetic peptides in place of a mixture of HIV antigens(17), thus improving specificity. The Western blot detects antibodies to the various structural proteins of the virus, the envelope proteins *(env)* gpl60, gpl20 and gp41, and the *gag* (p55, p40, p24, pi 7) and the *pol* (p66, p51 and p32) proteins. The current WHO criteria requires the presence of at least 2 of the 3 *env* proteins gpl60, gpl20 and gp41(18), whereas the CDC criteria requires the presence of at least 2 of the 3 proteins p24, gp41 and gpl60/120(19). Since Western blot assay is expensive, 2 successive ELISA tests, using different antigens and/or different principles if positive, is considered sufficient for the diagnosis of HIV infection.

The problem occurs in the early diagnosis of babies infected through their mothers. Here, the usual antibody tests detect passively acquired antibodies of the IgG class. These antibodies persist for an average period of 15 months, but on occasion persist for longer periods(3). Complicating the issue further is the fact that antibodies may be completely undetectable in a minority of children with AIDS(3).

Many methods have recently been developed to improve the sensitivity and specificity of early diagnosis. Anti-HIV antibodies of the IgM and TgA class, which are produced by the infant in response to infection have been attempted to be detected. Because the IgM response is transient, and its detection is hampered by the presence of large quantities of maternal antibodies, this approach has not proved very useful. Detection of antibodies of the TgA class has shown greater promise, and the current techniques used are relatively sensitive and inexpensive(20). The limitation is that this technique fails to detect infection in more than 50% of babies under 3 months of age(21), though detection rates at later ages are better. Attempts at detection of the infant's IgG antibodies have been made by *in vitro* culture of lymphocytes(22).

Tests for viral antigens usually detect the p24 or the "core" antigen in the plasma. These tests though very specific, are too insensitive to identify a substantial proportion of infected infants during the first year of life(23). This is because of maternal antibody excess, resulting in the formation of antigen-antibody complexes. The use of acid hydrolysis to dissociate these complexes, results in increased sensitivity of the test(24). The p24 antigen levels, however, correlate with the viral burden, and thus the stage of the disease, and can thus be used as a marker for disease progression.

As with all other infectious diseases, culture of the virus is the most perfect method of diagnosis. A great deal of success has been achieved, and with current techniques, isolation rates of nearly 100% have been achieved in adults. In perinatally infected babies, success rates are lower, and in addition, negative cultures do not necessarily indicate the absence of infection(25). This could be because of a low viral load in some babies due to "late" infection during the peripartal period. Even more intriguing are a subset of infants who have positive viral cultures at birth, but subsequently become culture negative

and seronegative, and remain immunologically healthy(3). Refinements in culture techniques have been the introduction of co-culture of peripheral blood monocytes, in which the yield is higher(24). By and large however, culture technique are far too expensive and cumbersome for routine u»e.

Detection of viral genetic material in the blood is done by the polymerase chain reaction (PCR). This exquisitely sensitive system can detect ultra-minute quantities of proviral nucleic acid sequences or viral RNA in peripheral blood cells. This test has redefined the accuracy of diagnosis of perinatally transmitted infections, permitting early interventions. However, as in culture, failure of this test in the earliest weeks to detect infection in some babies subsequently proven to be infected has raised some questions(26). Again a subset of infants have been found who are initially PCR positive, but later become negative, and remain healthy.

With viral culture and PCR, the sensitivity of detection of infection is nearly 100% by 6 months and age, but these tests are not widely available. In many infants however, a diagnosis can be made by the clinical status and routine immunologic tests if the mother is known seropositive. Based on clinical symptoms, hyperimmunoglobinemia and low CD4/CD8 ratios, diagnosis could be made with 48% sensitivity and 100% specificity at the age of 6 months in one series(3).

Thus, though no single test is infallable in the earliest weeks, PCR and viral cultures approach closest to perfection. After the age of 3 months, detection of specific IgA is diagnostic, and at later stages, clinical features and routine immunologic tests are adequate.

The Evolving Natural History

Roughly about a third of infants who test positive at birth will ultimately be infected and will develop the disease. However, there are a group of infants who test positive by the most sensitive tests like viral culture and PCR at birth, but subsequently remain healthy(3). This group of infants, then, definitely overcome their infection; whether they do so by their own immunity or otherwise is not known.

Perinatally infected children present in a continuous spectrum in 3 broad groups with regard to their symptoms. The first group consists of infants in whom the diagnosis of AIDS is made before the age of 6 months, and in them, survival beyond the age of 1 year is unusua1(27). In this group, severe encephalopathy and opportunistic infections are the usual features. In the second group, symptoms develop after the age of 1 year, and manifestations include failure to thrive, recurrent bacterial infections and lymphoid interstitial pneumonitis. The third group are the long term survivors (LTS) and the natural history in them is just being unfolded(28). Earlier studies had tended to show that only minor illnesses were associated with LTS(29), but current data indicates that a wider range of clinical features may manifest. Ten per cent of LTS are completely asymptomatic, 30% have minor manifestations but 30% have diseases severe enough to meet AIDS definition criteria(28). Growth failure, fever, diarrhea neurologic dis-

ease and secondary infections, though seen more commonly in early disease, does not necessarily prevent LTS. Positive predictors of LTS include parotitis and lymphadenopathy which are part of the diffuse infiltrative lymphocytosis syndrome (DILS), associated with a milder form of the disease in adults and are possibly due to genetically determined immune responses to HIV(30). A proportion of perinatally infected children may thus live long enough and present with disease in adolescence.

What influences the rate of progression of the disease is not known with certainy, and only some factors that cause LTS is some children and rapid death in others are understood. There is evidence to suggest that those babies who are infected early in pregnancy have a more accelerated course of the disease than those infected late or during delivery(31). Other factors influencing outcome include genetic and immunologic host factors(30) and virus phenotype(32). Breastfeeding may delay onset of the disease during the first five years(33).

Grey Areas in Drug Therapy

Currently there are 3 antiretroviral agents that are used for the treatment of HIV infection, zidovudine (ZVD), dideoxyinosine (ddl, didanosine) and dideoxycytidine (ddC or zalcitabine). Many clinical trials with these drugs, singly or in combination or with other supporting drugs, with varying dosing schedules and time of starting therapy are in progress(34), bearing testimony to the vast gaps in our knowledge about the ideal therapy. All these trials must be viewed against the backdrop of the fact that direct measures of efficacy are difficult, and the starting and endpoints of these trials rely heavily on surrogate markers of disease progression. Since CD4+ cell counts had been shown to correlate with the stage of the disease, these counts have been the most frequently chosen markers in most trials. We now know that CD4+ cell counts are hot the most ideal markers, and are not effective substitutes for clinical endpoints, and this could perhaps be responsible for the widely divergent results of different trials. There is also no uniform agreement about the most appropriate method for adjustive CD4+ counts for age, and data published by the CDC, which gives estimates for median absolute CD4+ cell counts for 4 age bands for the first 72 months of life are the best available(35). Notwithstanding these facts, CD4+ cell counts remain the only practical method to assess disease progression.

Early studies had clearly shown the beneficial effects of ZVD when given parenterally(36) or orally(37) in ameliorating signs and symptoms in children with AIDS. The initial euphoria of ZVD use was, however, short lived, and now it is known that resistant strains of HIV can emerge rapidly within 6 months of therapy. The beneficial effects are thus short lived (38). Based on overall observations, some generalized conclusions about drug resistance of HIV can be made: *(a)* ZVD resistant strains emerge with prolonged therapy, *(b)* ZVD resistance increases with lower initial CD4+ cell counts and in advanced stages of the disease, (c) increasing ZVD resistance occurs with temporal and cumulative development of specific mutations

in the reverse transcriptase gene, and *(d)* resistance can be observed *in vitro* to all nucleoside inhibitors evaluated to date(39). The clinical significance of ZVD resistance in children is imperfectly understood, firstly because of the unpredictable nature of the disease in children, and secondly because of the lack of perfect markers for disease progression. CD4+ counts increase and serum p24 antigens levels and plasma HIV titres decline after initial therapy, only to rebound within a few months. These observations are, however, tainted by the fact that CD4+ cell counts have a natural decline during the first few years of life, and this is an important confouding variable in any analysis of pediatric data.

There is a general consensus that early antiretroviral therapy is beneficial and will halt the progression of the disease. Concorde trials(41), however, puts this issue under a cloud, since this study in adults clearly demonstrates that early therapy has no benefits "as compared to deferred therapy.

Combination therapy of ZVD with ddl or ddC was a logical outcome and was started with a view to reducing the toxicity of individual drugs and to delay the emergence of resistant strains. It was initially seen as a major advance and many trials of ZVD together or sequentially with ddl and ddC, or monotherapy with one of the three are on. Current data however has failed to show that combination therapy is either more effective or safer than monotherapy(41). At present, the treatment of HIV infection is limited by the lack of therapeutic alternatives, and viral resistance is a major limiting factor in the efficacy of the available drugs. It is hoped that in, the coming few years, substantial cumulative data would be accumulated and a consensus would be reached on the ideal treatment protocol.

A substantial amount of standardization has, however, been brought about in the symptomatic management of HIV infection in children, and in the management of their complications. The prophylaxis of *Pneumocystis carinii* pneumonia has been standardized(35) and the diagnosis and management of other opportunistic infections have been established. Of particular interest to our country have been the development of low cost treatment protocols, based on a "syndromic" approach, and adaptations of these modules for Indian conditions are being made by the National AIDS Control' Organization, Delhi. We have, in fact, been using similar treatment protocols in an adolescent group with AIDS, with gratifying results. However, since the total number of cases of pediatric AIDS is still low in India, it is imperative that data from different centres be pooled for meaningful results.

Conclusions

Considering that ATDS as a disease is only a little more than a decade old, a great deal has been known about the causative agent, the epidemiology and the clinical illness. The molecular biology of the virus, its targets and its immunology are being understood. A remarkable amount of data and information has been genereated over the last 5 years, but as yet the pieces of the puzzle do not seem to have fallen into place. For instance, a vaccine for prevention and a drug for durable cure is still elud-

ing science. It is hoped that in the years to come, an effective solution will emerge through worldwide efforts.

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