CHAPTER VI

SUMMARY
&
CONCLUSIONS
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Cytogenetic Findings in 222 Mentally Retarded Patients

Down Syndrome made the greatest contribution to mental handicap due to any chromosomal aberration. 116 (52.3%) of the total cases were confirmed to have DS, out of which 113(97.4%) were due to free trisomy and 4(3.5%) were due to Robertsonian translocation. Family studies revealed two cases of translocation to be \textit{de novo} and two cases to be of maternal origin. Of the remaining 106 cases, 104 were found to have normal chromosome complement while two patients were observed to have 46,XX,\textit{dup16q}\,\textit{13→?qter} and 46,XX,\textit{der(18pter→18q21.3::15p13→15pter}).

Six (2.7%) children were observed to have normal chromosome variants. Inversion Y was observed in 3(2.2%) out of 138 male children which is significantly higher when compared to normal population. All the 3 children had inherited the inverted Y from the father. Two trisomy 21 cases showed 15p+ and 22p+ variant which was inherited from the mother. A significantly higher number of males were observed in Down Syndrome cases when compared to the sex ratio of the mentally retarded children. The unique cases observed in the present study are:

Case 1 - 46,XX,\textit{t(21;21),inv9qh}
Case 2 - 47,XY,+21;\textit{t(11;21)}(11pter→11q13.1::21p12→21pter;21qter→21p12::11q13.1→11qter)
Case 3 - 46,XX,\textit{dup16q}\,\textit{13→?qter}
Case 4 - 46,XX,\textit{der(18pter→18q21.3::15p13→15pter})

To the best of our knowledge Case 1 and Case 3 are first reports of such cases.
Cytogenetic Findings in Parents of Free Trisomy 21

Out of the 128 parents of 64 DS children confirmed to have free trisomy 21, polymorphism was observed in either parent in 14 families. Eight (12.5%) cases of polymorphism were observed in fathers and in six (9.4%) cases in the mother. Inv9qh was observed in 6 families. The frequency of inv9qh was observed to be significantly higher in parents of DS on comparing with the incidence of inv9qh in control population. The frequency of invY in 64 paternal karyotypes was also significantly higher to that of normal population. Polymorphism was present in 10.9% of the families considering the 128 subjects (64 couples). Parental origin studies on the 10 informative families revealed that in 7 families non-disjunction had occurred in the parent carrying the variant chromosome. Spontaneous abortion studies also revealed higher frequency of fetal loss in families where one of the parent had a variant chromosome (27.5%) as compared to controls (7.41%) and the total families of free trisomy 21 (17.11%). Taking into account the higher frequency of variants observed in parents of DS and the results revealed by parental origin as well as analysis of spontaneous abortions in them, an interchromosomal effect is suggested, which results in a trisomic offspring.

Acrocentric Chromosome Association in Down Syndrome

NOR associations were analysed in DS children and controls. In both the subject groups D-G, D-D, G-G and multiple acrocentric chromosome associations were observed. A significantly higher association was observed in DS children as compared to controls. These results are in accordance with previous reports of increased satellite associations in parents of DS children. Thus, a relationship between increased
association and non-disjunction is suggested.

**Telomeric Association in Down Syndrome**

Telomeric associations were analysed in DS children and age matched controls. The single chromatid and double chromatid telomere to telomere associations were both found. The chromosomes were involved in random manner in the associations and practically all the chromosomes were observed in the associations. In both the subjects, telomeric associations were found to involve single chromatids in majority of the cases. The total frequency of telomere association was significantly higher in the DS children as compared to the age matched control children. There are a number of previous studies reporting on a possible relationship between telomeric associations and onset of carcinogenesis. Telomeric loss has also been reported in ageing of cells *in vivo* and *in vitro*. Further, it has long been established that DS children have a high risk for leukaemia and have precocious ageing. Thus, it is suggested that the significant increase of telomeric associations present in the DS children when compared to age matched controls seem to increase their risk for predisposition to leukaemia and precocious ageing.

**Parental Origin of the Extra Chromosome in Trisomy 21**

The parental origin of the extra chromosome and the meiotic stage of non-disjunction was carried out in Down Syndrome families. The results are in accordance with the previous reports. Maternal non-disjunction accounted for 83.8% cases and paternal non-disjunction for 16.2% cases. Non-disjunction occurred due to error in first meiosis in 80.9% cases and due to second meiotic error in only 19.1% cases. The
results showed that majority of errors occurred in young mothers who were below 35 years. Analysis of parental origin according to meiotic error and parental ages did not reveal any strong association of maternal or paternal age effect for Down Syndrome. Hence, it could be concluded that in majority of the cases, non-disjunction occurred in the mother and in the first meiotic division. The role of maternal age in the non-disjunction of chromosome 21 is indicated to be doubtful.

Mitotic Cell Division Errors in Mothers of Down Syndrome:

The observations made on chromosome displacement showed that there was a significant increase both for the number of cells with displacement and total number of chromosomes displaced in mothers of DS in comparison to control mothers. The frequency of involvement of individual chromosomes showed a high degree of significant correlation of chromosome displaced, so that smaller chromosomes were observed to be more frequently involved in displacement than the larger ones in both the subject groups. Moreover, mothers of DS showed a slightly higher frequency of displacement for chromosomes 21 and 22. Increased displacement and the shift in displacement towards the smaller chromosomes observed in mothers of DS is similar to that in controls which are exposed to conditions unfavourable for spindle polymerization, reported previously by few workers. Thus, the observations of displacement analysis suggests that some mothers of DS have altered spindle microtubular function which results in frequent failure of chromosomes to attach to the spindle fibre.

Anaphase lagging studies were carried out in both the subject groups to determine the frequency of the lagging chromosomes/chromatids A significantly higher
number of cells with lagging and total chromosome/chromatid lagging was observed in mothers of DS than in controls. Moreover, the frequencies of lagging were highly reduced in both groups on comparing with the displacement rates, which suggest that retrieval of the displaced chromosome is a common phenomenon in both the subject groups. However, significantly higher incidence of chromosome or chromatid lagging in mothers of DS indicate that retrieval of the displaced chromosomes is less efficient in them as compared to controls. The increased lagging frequencies could also be correlated with increased displacement rates in these individuals.

The results of the micronucleus analysis in cytochalasin B induced binucleates showed that micronuclei were observed in all the subjects (control mothers and those of DS), suggesting that elimination of the lagging chromosome in micronucleus is a common phenomenon. Significantly higher frequencies of the binucleates with micronuclei and total number of micronuclei were observed in mothers of DS than in controls which could be correlated to higher lagging frequencies in these individuals. The micronucleus analysis was also carried out without any treatment indicating that the elimination of lagging chromosomes/chromatids in the present study might be due to spindle failure.

In the aneuploidy analysis, significantly higher frequencies of aneuploidy were observed in mothers of DS in comparison to controls which could be an outcome of increased levels of displaced chromosomes in these individuals. The relative involvement of chromosomes in displacement and aneuploidy are similar, so that smaller chromosomes were more frequently involved in aneuploidy than the larger chromosomes. Thus, it is likely that displacement is an early step in predisposing to aneuploidy. The observation of hyperploid cells only in mothers of DS suggests that
random segregation in these subjects is of common occurrence. It is known through previous reports that elimination occurs most frequently when cellular conditions favour polymerisation and random segregation occurs most frequently when the conditions are unfavourable. Moreover, a number of studies have reported that factors affecting non-disjunction operate similarly in the mitotic and meiotic cells. Thus, it is concluded that cellular conditions in mothers of DS are less favourable for spindle polymerisation than the cells of control mothers.

**Spontaneous Abortions in Mothers of Down Syndrome**

Reproductive histories of mothers of free trisomy 21 and controls were analysed. Frequency of spontaneous abortions was significantly higher in mothers of DS than controls. Rates of spontaneous abortions were highest in the first trimester. In mothers of DS maximum number of abortions occurred at the maternal age 26-30 years while no abortion was reported in the age group above 36 years. The data indicates that in older women, the ability to selectively abort aneuploid fetuses is decreased. A significant increase was observed in the frequency of spontaneous abortions prior to the birth of index child as compared to the rates subsequent to the birth of index child, which might indicate the risk of having aneuploid child. Thus, the observations suggest a risk for an aneuploid child in young mothers of DS with high frequency of spontaneous abortions.
Half of knowledge is knowing where to find it.