Possible risk factors for Down syndrome and sex chromosomal aneuploidy in Mysore, South India

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BACKGROUND: Down syndrome (DS) and sex chromosomal aneuploidy (SA) are common chromosomal anomalies causing congenital malformations and mental retardation in humans. The well-established risk factor, advanced maternal age, was not found in many of the DS and SA cases in India, while the other possible risk factors have not been well studied. In view of this, the present study has been made.

MATERIALS AND METHODS: During the last 5 years, 150 clinically suspected DS and 25 SA cases were referred to our laboratory for chromosome investigation from major hospitals of Mysore city. Chromosome preparations were made from these patients after informed consent was obtained. Well-spread G-banded metaphase plates were analyzed by automated LEICA KARYO software. Two hundred and 100 randomly selected families belonging to different religions were used as controls for the DS and SA cases, respectively. Statistical analysis was carried out using logistic regression

RESULTS: Out of the 150 cases of DS, 122 had free trisomy 21, two were mosaic trisomy 21, and one had translocation. Logistic regression of case-control study of DS children revealed that the odds ratio of uncle-niece marriages, or second cousin marriages, or parents lived in rural region, or exposure of the parents to chemicals, or parents education status, or habits (tobacco/ alcohol used) of father, or mother not undergone prenatal scanning, or mothers with previous abortions were significant when all the variables of that category were used one at a time. Exposure of the parents to chemicals, parents' educational status, habits (tobacco/alcohol use) of the father, mother not undergone prenatal scanning, and history of previous abortions were significant when all the variables of that category were used one at a time. Similarly, except for consanguinity, history of previous abortions, and mother not undergone prenatal scanning, all other factors showed significant odds ratios in SA cases.

CONCLUSION: Besides the known risk factors, consanguinity, region (rural/urban) of residence of parents, exposure of parents to chemicals, educational status of parents, habits of father, prenatal scanning, and reproductive performance of mother are possible risk factors for chromosomal aneuploidy.

Key words: Down syndrome, possible risk factors, sex chromosomal aneuploidy, South India

Introduction

Chromosomal abnormalities are remarkably common in human reproduction. Meiotic nondisjunction is the major mechanism responsible for the majority of aneuploidies in early embryos.[1] Down syndrome (DS), the most frequent autosomal aneuploidy, causes mild to moderate mental retardation and occurs in one out of 600-800 live births, with an extra chromosome 21 due to nondisjunction.[2,3] Turner syndrome and Klinefelter's syndrome are common sex chromosomal aneuploidies (SA), which occur as a result of the absence of one X chromosome (45, XO) or the presence of an extra X chromosome (47, XXY), respectively; the prevalence is 1 in 3000 female newborns and one in every 500 to 1000 newborn boys, respectively.[4,5]

There are several reports indicating that most of the aneuploidies that are maternal-age dependent are generated during meiosis I of oogenesis.[6-9] It has also been shown that older men produce more sperm with aneuploidy.[10,11] Although the relation of advanced maternal age to an increased risk of DS has been established, there are few studies on the effects of other risk factors.[12-14] Though tremendous work has been done on DS and SA since 1959, most of the

results obtained on etiological and demographic factors were based on Western data. The situation in India is entirely different; it has a large and extremely diverse population consisting of more than 4000 culturally and anthropologically well-defined populations with little gene flow between them. Myriads of castes, subcastes, and tribes, with a high degree of endogamy and consanguinity in various sects, along with a population of more than one billion, provides an excellent opportunity for birth defect investigation. In view of this, here we report the other possible risk factors for DS and SA in Mysore, South India.

Materials and Methods

Chromosomal aneuploidy cases

During the last 5 years, 150 clinically suspected DS and 25 SA cases were referred to our laboratory for chromosome investigation from major hospitals of Mysore city. Peripheral blood samples were collected from these patients and subjected to leucocyte culture following the method of Moorhead *et al*. (1960). G-banding was performed by a modified method of Seabright (1971). Well-spread G-banded metaphase plates were scored and analyzed by automated LEICA KARYO software. Informed consent was obtained from the parents of the patient before inclusion in the study.

Control population

Randomly selected healthy families belonging to different religions and different localities in and around Mysore city, South India, were used as controls; there were 200 control families for DS and 100 families for SA. To generate case-control data set for DS, one child was selected randomly from each of the 200 control families; similarly, for SA cases, one child was randomly selected from each of the 100 control families used. A genetic register was established by collecting complete information on the patients and control families. With this information, the pedigree of the families under study was constructed.

Statistical analysis

Logistic regression was performed using the software SPSS, version 10.0, to record the effect of the variables. Case-control status was used as the dependent variable and consanguinity, habits, region of residence, exposure to chemicals, educational status of parents, reproductive performance of mother, and prenatal scanning as the covariates. Results were reported as odds ratio from model with one variable at a time.

Results

Out of 150 cases of DS, 122 had free trisomy 21, 2 had mosaic trisomy 21, and one had translocation. A total of seven different possible risk factors were assessed in 200 control and 150 DS families as well as in 100 control and 25 SA families. Figures 1 and 2 show the results of 18 assessments done of seven possible risk factors for DS and SA. A noticeable difference was found between cases and controls with regard to risk factors, such as region of residence, educational status of parents, habits of father, and also reproductive performance of mother.

Table 1 shows the results of the univariate logistic regression in the case-control study of DS children. The relationship of the seven risk factors with DS was analyzed by calculating the odds ratios (with the 95%
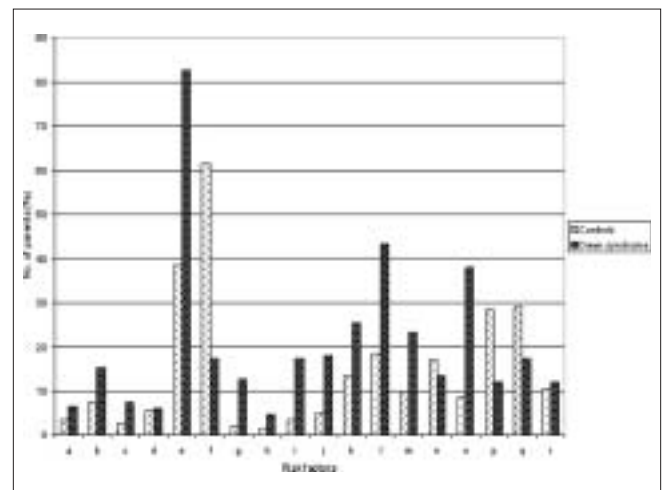
Figure 1: Distribution pattern of seven risk factors in control (200) and Down syndrome (150) families. Consanguinity, a = first cousin, b = uncle-niece, c = second cousin, d = far relatives; Region of residence, e = rural, f = urban; Exposure of parents to chemicals, g = father exposed to chemicals, h = mother exposed chemicals, I = father and mother exposed to chemicals; Educational status of parents, j = father uneducated, k = mother uneducated, l = both uneducated; Habits of father, m = father smoker, n = father alcoholic, o = father smoker and alcoholic; Prenatal scanning, p = mother not undergone prenatal scanning; Reproductive performance of mother, q = mother with abortions, r = mother with still births

confidence intervals)

. When each variable was examined separately, the odds ratios of the following factors were found to be
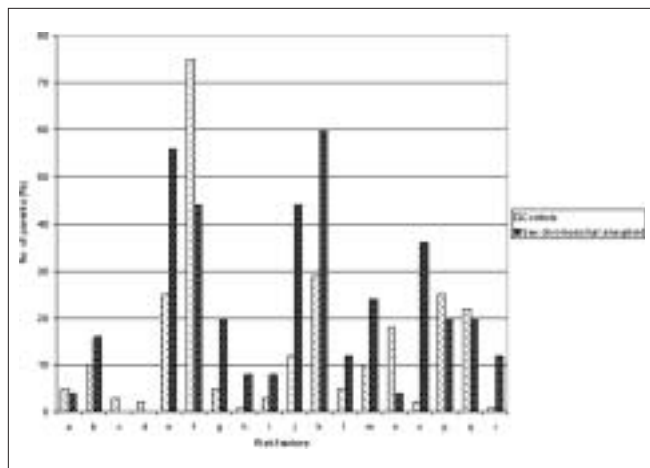
Figure 2: Distribution pattern of seven risk factors in control (100) and sex chromosomal aneuploidy (25) families. Consanguinity, a = first cousin, b = uncle-niece, c = second cousin, d = far relatives; Region of residence, e = rural, f = urban; Exposure of parents to chemicals, g = father exposed to chemical, h = mother exposed chemicals, i = father and mother exposed to chemicals; Education status of parents, j = father uneducated, k = mother uneducated, l = both uneducated; Habits of father, m = father smoker, n = father alcoholic, o = father smoker and alcoholic; Prenatal scanning, p = mother not undergone scanning; Reproductive performance of mother, q = mother with abortions, r = mother with still births

statistically significant: a) residence in rural area, b) uncle-niece and second cousin marriage, c) exposure to chemicals of the father or both the parents, d) educational status of parents, e) habits of father such as smoking/smoking plus alcohol use, f) past history of frequent abortions in the mother, and g) no prenatal scanning done.

Table 2 gives the results of the univariate logistic regression in the case-control study on SA children. Odds ratios (with 95% confidence intervals) were calculated to assess the relationship of each of the seven risk factors with SA: a) region of residence, b) father exposed to chemicals, c) educational status of parents, d) habits of father like smoking plus alcohol use, e) a past history of many still births in the mother.

Discussion

Advanced paternal age combined with advanced maternal age significantly influences the incidence of Down syndrome.[15] There are contradicting reports regarding the maternal and paternal ages that increases the risk for chromosomal aneuploidy in India.[16-18] Our earlier findings in Mysore, South India,[19] had revealed that 75% of DS children were born to young

Table 1: Comparison of risk factors between controls (200) and Down syndrome (150) families in the Mysore population, along with logistic regression analysis (C.I. = confidence intervals)

Risk factors	Variables	Univariate	
		Odds ratio (95% C.I.)	P value
Consanguinity	First cousin	1.969 (0.732 to 5.301)	0.180
	Uncle-niece	2.234 (1.122 to 4.447)	0.022*
	Second cousin	3.086 (1.049 to 9.079)	0.041*
	Distant relatives	1.097 (0.443 to 2.7180)	0.842
Region of residence	Urban	0.131 (0.079 to 0.219)	0.131
	Rural	7.618 (4.576 to 12.682)	0.0001*
Exposure to chemicals	Father	7.106 (2.364 to 21.361)	0.0001*
	Mother	3.213 (0.817 to 12.637)	0.095
	Father and mother	5.828 (2.455 to 13.837)	0.0001*
Uneducated member	Father	4.166 (1.948 to 8.907)	0.0001*
	Mother	2.174 (1.257 to 3.759)	0.0001*
	Father and mother	3.409 (2.105 to 5.519)	0.0001*
Habits of father	Alcoholic	0.708 (0.386 to 1.299)	0.265
	Smoke	2.638 (1.448 to 4.805)	0.002*
	Both	5.382 (2.948 to 9.825)	0.0001*
Prenatal scanning	Mother not undergone prenatal scanning	3.42 (0.191 to 0.611)	0.0001*
Reproductive performance	Abortions	0.514 (0.305 to 0.865)	0.012*
	Still births	1.162 (0.596 to 2.268)	0.659

*Significant

Table 2: Comparison of risk factors between controls (100) and sex chromosomal aneuploidy (25) families in the Mysore population, along with logistic regression analysis (C.I. = confidence interval)

Risk factors	Variables	Univariate	
		Odds ratio (95% C.I.)	*P* value
Consanguinity	First cousin	1.96 (0.732 to 5.301)	0.180
	Uncle-niece	2.234 (1.122 to 4.447)	0.22
Region of residence	Urban	0.262 (0.105 to 0.651)	0.04*
	Rural	3.818 (1.537 to 9.488)	0.04*
Exposure to chemicals	Father	4.749 (1.256 to 17.959)	0.022*
	Mother	8.608 (0.748 to 99.05)	0.084
	Father and mother	2.811 (0.444 to 17.810)	0.272
Uneducated member	Father	5.769 (2.133 to 15.564)	0.001*
	Mother	3.672 (1.479 to 9.117)	0.005*
	Father and mother	2.591 (0.575 to 11.665)	0.215
Habits of father	Alcoholic	0.190 (0.024 to 1.498)	0.115
	Smoke	2.842 (0.921 to 8.767)	0.069
	Both	27.56 (5.450 to 139.38)	0.0001*
Prenatal scanning	Mother not undergone prenatal scanning	1.33 (0.453 to 3.923)	0.602
Reproductive performance	Abortions	0.886 (0.299 to 2.632)	0.828
	Still births	6.681 (1.053 to 42.406)	0.044*

*Significant

mothers, whose ages ranged from 18-29 years. The age distribution of the mothers as well as of the fathers of DS cases and controls indicates that both maternal and paternal age have no decisive influence on the manifestation of DS. Logistic regression analysis revealed that the age of a maternal grandmother at the time of the birth of the mother is a risk factor for the occurrence of DS.[19] Here, we report the influence of other possible risk factors for chromosomal aneuploidy.

Consanguinity

The incidence of malformations was high in consanguineous marriages as compared to nonconsanguineous marriages in India.[20,21] In the present study, the logistic regression analysis for consanguinity, using all four covariates, have shown that when these covariates were considered one at a time, the effect of a consanguineous marriage to, for example, a first cousin or a distant relatives was diluted; however, still the effect of uncle-niece and second-cousin marriages were not diluted showing an increase in odds ratio by 23% and 8% respectively. This supports the fact that consanguineous marriages between uncle and niece and between second cousins are risk factors for the manifestation of DS. However, consanguinity in India is also influenced by population structure and sociocultural, ethnic, religious,

and economic factors. In SA cases, consanguinity is not a significant risk factor; this finding could be due to the rarer occurrence of SA than DS.

Rural or urban residence

Various studies have reported that the frequency of congenital malformations is more in babies born to rural mothers than urban mothers.[20,22,23] In the present study, more DS and SA children were born in rural areas than in urban areas. Logistic regression analysis using these as two covariates showed that when they were considered separately, the effect of urban residence was diluted but not that of rural residence, which showed an increase in odds by 61%. Logistic regression for SA revealed that the effects of both the covariates were nonsignificant. However, this could be due to inadequate antenatal care and poor medical awareness in the early stages of pregnancy in the rural areas. Various factors, such as low socioeconomic status, environmental factors, and nutritional deficiency, could contribute to the increased incidence of malformations in rural areas. These studies indicate that medical awareness is essential for every parent, whether they belong to urban or rural regions.

Exposure to chemicals

Environmental toxins and drugs have the potential

for inducing chromosomal nondisjunction.[22,24,25] In the present study, neither father nor mother was exposed to toxic chemicals, but they were exposed to drugs used for treatment of convulsions, diabetes, high blood pressure, and other chronic diseases. Logistic regression analysis using all the covariates have shown that when these covariates were considered one at a time, the effect of mother's exposure to chemicals was diluted but the effect of the father's, as well as both parents', exposure to chemicals was not diluted, showing an increase in odds by 10% and 82%, respectively, in DS. In SA cases, the increase in the odds ratio was 74% when the father had been exposed to chemicals. This analysis supports the hypothesis that exposure to chemicals is a possible risk factor for chromosomal aneuploidy.

Educational status of parents

Various investigations have reported the strong relationship between education of the mother and the use of the health services for prenatal diagnosis.[26,27] In the present study, logistic regression showed that when education of mother, education of father, and education of both parents were considered one at a time, none of the variables were diluted, showing an increase in odds by 16%, 17%, and 40%, respectively. Educational status of parents is also important in the case of SA. Therefore, education appears to be an essential component in helping the parents be aware regarding pregnancy care and the health of their progeny.

Habits of the parents

There are contradictory reports on the effect of cigarette smoking and consumption of alcohol on the occurrence of chromosomal aneuploidy.[28-32] Generally, in India, smoking and alcohol use is unusual among mothers. In the present study, three common habits among the fathers - tobacco smoking, drinking of alcohol, and both alcohol *and* smoking - were found to be high in DS families. Logistic regression analysis using all the covariates have shown that when these covariates were considered one at a time, the effect of alcoholism in the father was diluted but the other effect of the other two covariates were not diluted, showing an increase in odds by 63% and 38%, respectively. Similarly, for SA, logistic regression revealed significant odds ratio when the father was both smoker

and alcoholic. Either alcoholism or smoking acting alone increased the risk for DS (showing significant odds ratios) but this was not so for SA, indicating that autosomal aneuploidies are more sensitive to the habits of parents.

Prenatal scanning

Several studies have documented the use of socioeconomic disparities, ethnicity, parity, and place of residence in prenatal diagnosis.[26,33,34] In the present study, although more number of mothers of DS children had undergone prenatal scanning than that of controls, the defect was not noticed. Generally, prenatal scanning was done only to screen for gross anomalies. Logistic regression analysis using the covariate has shown that when the covariate was considered one at a time, the effect of mother not having undergone prenatal scanning was not diluted, showing an increase in odds by 42%. The number of mothers who had undergone prenatal scanning was the highest in SA families, which suggests that scanning is not an appropriate method to detect SA. Therefore, proper prenatal diagnosis has to be implemented in all the health-care centers both in rural and urban areas.

Reproductive performance of mother

The major issues in women's reproductive health are fertility regulation, abortion, maternal mortality, sexually transmitted diseases, and infertility. An increased risk of aneuploidy is present in women who have had many spontaneous abortions.[35] Maternal health and reproductive potential have a prominent etiological significance in the occurrence of DS.[36] In the present study, the control mothers had more number of abortions than the mothers in DS and SA families. This finding supports the hypothesis that spontaneous abortions reduce the risk for chromosomal nondisjunction. Logistic regression analysis using all the covariates have shown that when these covariates were considered together the effect of mothers reproductive performance, like mothers with abortions, was not diluted, showing an increase in odds by 51%. For SA, the odds ratio was significant for still births.

During the present study, the following limitations were encountered. The information pertaining to parents was recorded during the data collection by interviewing

the family members. Although controls were selected randomly in different locations of Mysore, and included all religious groups, this selection cannot be absolute because some of the families did not give consent for investigations. These findings can be applied to the families with large progenies in India or elsewhere. Thus, besides the known risk factors, consanguinity, region of residence of parents, exposure of parents to chemicals, educational status of parents, habits of father, prenatal scanning, and reproductive performance of mother are all potential risk factors for the manifestation of chromosomal aneuploidy.

Acknowledgments

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