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THE HOSPITAL BASED CYTOGENETIC STUDY OF FREQUENCY OF CHROMOSOMAL ABNORMALITY IN OPD PATIENTS WITH SUSPECTED GENETIC DISORDERS

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ABSTRACT

Background: Chromosomal abnormalities are remarkably common in human reproduction. Meiotic non disjunction is the major mechanism responsible for the majority of aneuploidies in early embryos. Down syndrome and Edward syndrome are autosomal whereas Turner syndrome and Klinefelter syndrome are common sex chromosome aneuploidies. **Aims and objective:**[1] To confirm chromosomal abnormality in OPD patients.[2] To assess the referral rate from hospital for suspected genetic disorders. **Methods and Material:** This observational study performed during Jan 2012 to Dec 2016 on OPD Patients with suspected genetic disorders referred to Genetic Lab of dept. of Anatomy. After informed consent was obtained, data regarding age, sex, clinical features, family history, socio-economical status and attitude towards disorders were recorded. Cytogenetic investigations were carried out using peripheral blood samples on all 838 referred cases. The standard Karyotyping method was used to confirm chromosomal abnormality. The collected data was compared with the globally accepted statistics of chromosomal abnormality. **Result:** The frequency of suspected patients with genetic disorder in 1000 referred patients was 1.88, 1.00, 0.45, and 0.26 from Pediatrics, Gynecology, Medicine and Surgery Department respectively where the total incidence was 0.73. Out of 838 referred cases 77 were of Down syndrome, 28 Turner Syndrome, 8 Kleinfelter syndrome and 3 Edward syndrome. **Conclusion:** Chromosomal abnormality is prevalent in the visiting patients of our hospital although the frequency rate still low as compared to published data of the other hospitals which may be due to low referral from of our region.

KEYWORDS: Down syndrome; Turner syndrome; Klinefelter syndrome; Edward syndrome; OPD patients.

INTRODUCTION

Chromosomal abnormalities are remarkably common in human reproduction. Meiotic non disjunction is the major mechanism responsible for the majority of aneuploidies in early embryos. Down syndrome and Edward syndrome are common autosomal aneuploidies. Down syndrome incidence is about 1 in 700 births in western population and in Indian population is 1 in 920 births[1] whereas the incidence of Edward syndrome occurs in about one in 6000- 8000 live births.[2] Turner syndrome and Klinefelter syndrome are common sex chromosome aneuploidies. Turner syndrome is estimated to affect about 3% of all female fetuses, only 1% of affected fetuses survive to term, leading to an incidence of 1 per 2500 live female births[3] and Klinefelter syndrome has prevalence 1 in 500 to 1000 newborn boys. There is a paucity of Indian data and a large number of patients of genetic disorder go undetected.

Our institute receives patients from Urban, Rural and Tribal areas. To assess the frequency of genetic disorders in OPD patients, this study was undertaken with an aim to know the frequency of the Genetic Disorders in patients visiting Pediatrics, Obstetrics and Gynecology (Ob.Gy.), Medicine and Surgery departments of the institute.

METHODS AND MATERIAL

Subject and Methods

This was observational study, conducted on OPD patients at our institute from Jan 2012 to Dec 2016 (Table 1). The OPD patients who were suspected to have chromosomal anomalies were referred to the Genetic Lab. of dept. of Anatomy (Table 2). The study protocol was presented before the Institutional Ethics committee and approval was obtained. The individuals with suspected chromosomal abnormalities were included and subjects with metabolic disorders and multi factorial genetic diseases were excluded. The informed consent was obtained, the age at referral, reason for the referral; clinical features and family history were recorded.

Cytogenetic investigations

Cytogenetic investigations were carried out on 838 referred cases of suspected cases. (Table 3) Peripheral blood samples were collected and subjected to leukocyte culture. 2ml PHA stimulated blood sample was cultured for 72 hours in RPMI-1640 medium supplemented with 20% qualified; heat inactivated fetal bovine serum, 100U/ml penicillin and streptomycin, without mitogen at 37°C. The culture was exposed to colchicine (10 µg/ml) for 90 minutes followed by hypotonic treatment by potassium chloride (0.075M KCL) for 20 minutes at 37°C. Then fixed in Methanol: Glacial acetic acid (3:1) and dropped on wet ice cold grease free slides. The chromosomes were G-banded with trypsin-giemsa banding. Olympus BX51 Research microscope was used to screen, capture and karyotype the metaphase chromosomes. The results interpreted according to International Standard Chromosome Nomenclature (ISCN). Diagnosis was done by Karyotyping, involving a study of at least 50 metaphases and including G banding. Simple descriptive statistical methods were used and percentage calculated wherever appropriate.

OBSERVATIONS AND RESULTS

It has been observed that a number patients referred from pediatrics dept. has the highest frequency of 1.8821 in 1000 referred patients, Ob.Gy. has the frequency of 1.0070 in 1000 referred patients, Medicine has the frequency of 0.4538 in 1000 referred patients, Surgery has the frequency of 0.2615 in 1000 referred patients and total frequency of 0.7378 in 1000 observed in OPD patients attending above departments (Table 2). The frequency of referred male patients is 36.6348 % whereas the frequency of referred female patients is 63.3651 %.

Table 1: Number of OPD Patients attending the Hospital in 5 years.

Sr. No.	Departments	Year 2012	Year 2013	Year 2014	Year 2015	Year 2016	TOTAL
01	Paediatrics	35929	39949	38319	39721	41069	1,94,987
02	Ob.Gy.	38864	42122	39964	40878	42723	2,04,551
03	Medicine	50057	68532	83460	87192	87506	3,76,747
04	Surgery	42177	79272	74197	81687	82059	3,59,392
				TOTAL			11,35,677

Table 2: No. of Patients referred from depts. To Genetic Lab. (Jan 2012 to Dec 2016).

Sr. No.	Dept.	No. of Male Patients	No. of Female Patients	TOTAL	Frequency of suspected patients of Genetic Disorder in 1000 referred patients
01	Paediatrics	160	207	367	1.8821
02	Obstetrics and Gynecology	00	206	206	1.0070
03	Medicine	94	77	171	0.4538
04	Surgery	53	41	94	0.2615
TOTAL		307	531	838	0.7378

Table 3: Report of Karyotyping.

Sr No.	Dept.	Normal Males	Normal Females	Down's Syndrome	Turner Syndrome	Kleinfelter Syndrome	Edward syndrome	Sex Reversal Male	Sex Reversal Female	Clumping of Chromosome	Contaminated Sample	TOTAL
1	Pediatric	139	122	62	18	0	2	2	2	05	15	367
2	Ob.Gy.	00	184	08	06	0	0	0	1	00	07	206
3	Medici	84	65	05	04	05	1	0	0	00	07	171
4	Surgery	49	37	02	00	03	0	0	0	00	03	94
5	Total	272	408	77	28	08	03	02	03	05	32	838

Table 4: Age Distribution in the Patients in Down Syndrome.

Depts.	Below 1 month		Below 1 Year		Below 5 Years		Below 10 years		Above 10 Years		TOTAL
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	
Paedia.	05	08	08	15	09	04	06	04	02	01	62
Ob.Gy.	Nil	Nil	Nil	01	Nil	02	Nil	01	Nil	04	08
Medicin	Nil	Nil	Nil	Nil	Nil	Nil	01	01	01	02	05
Surgery	Nil	Nil	Nil	Nil	Nil	Nil	00	Nil	01	01	02
Total	05	08	08	16	09	06	07	06	04	08	77
% of referral	15.15	18.18	24.24	36.36	27.27	13.63	21.21	13.36	12.12	18.18	

Table 5: Age Distribution in the Patients in Turner Syndrome.

Depts.	Below 5 years	Below 10 Years	Below 15 Years	Above 15 Years	TOTAL
Paediatrics	2	3	8	5	18
Ob.Gy.	Nil	Nil	3	3	06
Medicine	Nil	Nil	2	2	04
Surgery	Nil	Nil	Nil	Nil	Nil
Total	02	03	13	10	28
% of referral	7.1428%	10.7142%	46.4285%	35.7142%	

Table 6: Age Distribution in the Patients in Kleinfelter Syndrome

Depts.	Below 5 years	Below 10 Years	Below 15 Years	Above 15 Years	TOTAL
Paediatrics	Nil	Nil	Nil	Nil	00
Ob.Gy.	Nil	Nil	Nil	Nil	00
Medicine	Nil	02	03	Nil	05
Surgery	Nil	Nil	01	02	03
Total	00	02	04	02	08
% of referral	00	25%	50%	25%	

Table 7: Age Distribution in the Patients in Edward Syndrome

Depts.	Below 5 years	Below 10 Years	Below 15 Years	Above 15 Years	TOTAL
Paediatrics	01	01	NIL	NIL	02
Ob.Gy.	NIL	NIL	NIL	NIL	00
Medicine	NIL	NIL	01	NIL	01
Surgery	NIL	NIL	NIL	NIL	00
Total	01	01	01	00	03
% of referral	33.33%	33.33%	33.33%	00	

Table 8: Incidence of Disease (per 1000) in Total Down's Syndrome, Turner Syndrome, Kleinfelter Syndrome and Edward Syndrome Patients attending the OPD.

Department	Total No. of Patient	DOWN Syndrome		Kleinfelter Syndrome		Turner Syndrome		Edward Syndrome	
		Patients Detected	Incidence in 1000 pts.	Patients Detected	Incidence in 1000 pts.	Patients Detected	Incidence in 1000 pts.	Patients Detected	Incidence in 1000 pts.
Paediatrics	1,94,987	62	0.31796	00	00	18	0.09231	03	0.015385
Ob.Gy.	2,04,551	08	0.03911	00	00	06	0.01955	00	00
Medicine	3,76,747	05	0.01327	05	0.01327	04	0.01061	00	00
Surgery	3,59,392	02	0.00556	03	0.008347	00	00	00	00
TOTAL	11,35,677	77	0.06780	08	0.007044	28	0.02465	03	0.002641

Table 9: Age of Parents in Detected cases of Down Syndrome, Turner Syndrome, Kleinfelter Syndrome and Edward Syndrome

Syndrome	Age Group / Parents	Below 20 years	Below 25 years	Below 30 years	Below 35 years	Below 40 years	Above 40 years	Total
Down Syndrome	Mother	3	25	27	12	8	2	77
	Father	0	9	32	21	8	7	77
Turner Syndrome	Mother	1	6	8	10	2	1	28
	Father	0	2	6	10	6	4	28
Kleinfelter Syndrome	Mother	0	1	2	2	2	1	8
	Father	0	0	1	2	3	2	8
Edward Syndrome	Mother	0	0	1	1	1	0	3
	Father	0	0	1	1	1	0	3

Table 10: History of parents for addiction.

Sr. No.	Addiction		Down Syndrome Total 77		Turner Syndrome Total 28		Kleinfelter Syndrome Total 08		Edward Syndrome Total 03	
			Mother	Father	Mother	Father	Mother	Father	Mother	Father
1	No of Cases	Non addict	42	12	14	09	04	03	02	01
	%	Addiction	54.5%	15.58%	50.00%	32.14%	50.00%	37.5%	66.66%	33.33%
2	No of Cases	Tobacco Chewing	28	20	08	08	02	02	00	00
	%	Tobacco Chewing	36.3%	25.97%	28.57%	28.57%	25.00%	25.0%	00	00
3	No of Cases	Smoking	03	16	02	07	01	02	00	00
	%	Smoking	3.89%	20.77%	07.14%	25.00%	12.5%	25.0%	00	00
4	No of Cases	Alcohol	00	10	00	02	00	01	00	00
	%	Alcohol	00	12.98%	00	07.14%	00	12.5%	00	00
5	No of Cases	Sr. No. 2 + 3	04	11	04	01	01	00	01	01
	%	Sr. No. 2 + 3	5.19%	14.28%	14.28%	03.57%	12.5%	00	33.33%	33.33%
6	No of Cases	Sr. No. 2+3+4	00	08	00	00	00	00	00	01
	%	Sr. No. 2+3+4	00	10.38%	00	00	00	00	00	33.33%

In Pediatrics out of 367 referred patients Normal males were 38.0821 %, Normal females 33.1506 %, Down syndrome 16.9863 %, Turner syndrome 4.9315 %, Kleinfelter syndrome 0.00% and Edward syndrome 0.5479 %.

In Ob.Gy. out of 206 referred patients Normal females 89.3203%, Down syndrome 3.8834%,Turner syndrome 2.9126%, Kleinfelter syndrome 0.00% and Edward syndrome 0.0%.

In Medicine out of 169 referred patients Normal males were 52.0710 %, Normal females 39.6449 %, Down syndrome 2.9585 %, Turner syndrome 2.3668 %, Kleinfelter syndrome 2.9585% and Edward syndrome 0.5917 %.

In Surgery out of 93 referred patients Normal males were 54.8387 %, Normal females 39.7849 %, Down syndrome 2.1505 %, Turner syndrome 0.00 %, Kleinfelter syndrome 3.2258% and Edward syndrome 0.5479 %.

DISCUSSION

Globally as of 2010, the incidence of Down syndrome is 1 in 1000 births and results about 17000 deaths. Down syndrome affects about 23000 – 29000 children born in India every year. Its frequency in tribal population was observed slightly higher.[4] The studies conducted on urban population of Mysore, Hyderabad, Baroda, Andhra Pradesh, are similar.[1, 5-7] In our study the sex ratio of 1.5:1 showed a male preponderance. Das et al.2015 reported the sex ratio 1.38:1 male preponderance.[8]

We could not find out the references for the referral age of the Down syndrome patients. In our study we find the referral age in Down syndrome of males patients are 15.15 % above 1 month, 24.24% below 1 year, 27.27% below 5 years, 21.21% below 10 years and 12.12% above 10 years. The referral age in female patients are, 18.18% above 1 month, 36.36% below 1 year, 13.63% below 5 years, 13.63% below 10 years and 18.18% above 10 years (Table 4).

India's birth rate is 22000 and an Incidence of Turner Syndrome is 1 in 2500 female births, about 5200 Turner Syndrome Girls are born in India each year.[3] In Western Word one fifth to one third are referred in new born period and one third in early childhood and10% in adult life. In India 9% were referred in early childhood, 3% in late child hood and majority are diagnosed between 11 to 20 years. In our study 7.14 % patients were referred below 5 years, 10.71% below 10 years,46.42% below 15 years and 35.71% above 15 years which co relates with the work of Athar et al. 2012[9] (Table 5). According to Maiti and Chatterjee 2014 the mean age at diagnosis was 12 years,[3] which matches with our study.

The incidence of Kleinfelter Syndrome is 1 in 500 to 1000 new born boys. In our study we find the referral age in male patients are, below 5 years, 00% below 10 years 25% and below 15 years 50% and above 15 years 25 % (Table 6). According to Morris, et al. 2008 the prevalence of the XXY has risen from 1.09 to 1.72 per 1000 male births (P = 0.023),[10] Zeuthen and Nielsen 1978 reported that 3840 males examined for military services the prevalence being 0.78 per 1000.[11]

The incidence of Edward syndrome occurs in about one in 6000- 8000 live births. In our study we find the referral age are, below 5 years, 33.33%, below 10 years 33.33%, below 15 years 33.33% and above 15 years 00 % (Table 7) According to Lal 2016, approx. 95% of conceptus with Trisomy 18 die as embryos or fetuses[2] For live born infants the estimated probability of survival to age 1 month was 38.6% and to age 1 year was 8.4%. Meyer et al. 2015 found a 5 year survival rate in 12.3%.Long term survival up to age of 27 years has been reported. Approximately 80% of Trisomy 18 cases occur in females.[12]

In our study the incidence of Down syndrome in 1000 patients referred to lab is over all 0.06780, which varies with different departments. In Turner syndrome the incidence in 1000 patients referred to lab is over all 0.02465, which varies with different departments. In Kleinfelter syndrome the incidence in 1000 patients referred to lab is over all 0.007044, which varies with different depts. In Edward syndrome the incidence in 1000 patients referred to lab is over all 0.002641, which varies with different depts. (Table 8)

In our study in Down syndrome we find 35.0641 % mother are below 30 years and 32.4675 % mother are below 35 years of age, where as we find 41.55 % father are below 30 years and 27.27 % father are below 35 years of age. (Table 9) According to Vunditi et al. 2011 a high frequency of DS births occurs in mothers of younger age groups (mean age 26 years).[13] Ghosh et al. 2011 quote that smokeless chewing tobacco was associated with significant risk for meiosis II nondisjunction and achiasmate (nonexchange) meiosis I error among young mothers. By contrast, the risk due to oral contraceptive pills was associated with older mothers. Study results suggest that the chewing tobacco risk factor operates independently of the maternal age effect, whereas contraceptive pill-related risk may interact with or exacerbate age-related risk. Moreover, both risk factors, when present together, exhibited a strong age-dependent effect.[14] According to Lakhan and Kishore 2014 younger maternal (mean age 30 years) was reported in tribal population, may be due to consanguineous marriages, high birth rate and advanced age of parents. Chemical exposure and second hand smoke may be contributing factors.[4] In our study we find 18 parents have consanguineous marriages. According to Isaac 1985 et al. a survey in Hyderabad gave an incidence of 1.17 per 1000 or 1 in 853 live births, with a significant increase in the mean maternal age and sex ratio was also higher.[6] The Sheth et al. 2007, shown increased number of DS babies born to the young mothers; this could either be due to MTHFR gene polymorphism and / or nutritional factor.[15]

In Turner syndrome we find 28.5714 % mother are below 30 years and 35.7142 % mother are below 35 years of age, where as we find 21.4285 % father are below 30 years and 35.7142 % father are below 35 years of age (Table 9). According to Hagman et al. 2012 more women with age (40 +) delivered girls with TS. 3.2 % compared with 1.8% in general population.[16] Athar et al. 2012 reported that out of 89 children 20 of the children were born out of consanguineous marriages.[9] We got history of 2 consanguineous marriages in this syndrome.

In Kleinfelter Syndrome we find 25.00 % mother are below 30 years and 35 years of age, where as we find 25.00 % father are below 30 years and 37.50 % father are below 35 years of age (Table 9). Morris et al. 2008 reported that increase in XXYs is the result of an increase in paternal, rather than maternal non disjunction. Around 50% of XXY are Maternal and 50% paternal in origin.[10] Maternal non disjunction results in an ovum with two X chromosomes, which theoretically to be fertilized by X or Y chromosome, resulting in equal frequency of XXY and XXX conceptions, which was not seen. The XXYs of paternal origin result from non disjunction of XY during paternal MI, XYYs result from non disjunction of at paternal MII.

In Edward syndrome we find the age of mother and father is 33.33 % in age group below 30 years, below 35 years and below 40 years. (Table 9) but more conceptions are affected by the syndrome because the majority of those diagnosed with the condition prenatally will not survive to birth. Although women in their 20s and early 30s may conceive babies with Edward syndrome, the risk of conceiving a child with it increases with a woman's age. The average maternal age for conceiving a child with this disorder is 32½.

In our study we have also recorded the history of addictions of parents referred for Karyotyping (Table 10). In Down syndrome 36.3% mother and 25.97%

father were addicted to Tobacco chewing, 20.77% father addicted to smoking and 14.28% were addicted to both. In Turner syndrome we find 28.57% mother and father are addicted to Tobacco chewing and 25% father for smoking and 14.28% mother were addicted to both. In Kleinfelter syndrome 25% mother and father were addicted to Tobacco chewing, 12.5% mother prone for smoking and 25% father prone for smoking. In Edward syndrome 33.33% mother and father are addicted to Tobacco chewing and smoking. Hence it appears that Tobacco may be playing a role in aneuploidies.

India is facing demographic shift to non-communicated diseases. Congenital malformation and genetic disorders are important causes of morbidity and mortality. With a very large population, high birth rate and consanguineous marriages and other ecological factors there is a high prevalence of genetic disorders in India. Due to inadequate diagnostics managements and rehabilitation facilities the burden of these disorders is greater than western countries. Genetic disorders received little attention from health services. Community control of common disorders like Down syndromes required high priority and genetic services should be integrated in to the existing primary healthcare and medical services and should be referred to tertiary care centres. Each medical college should have a Genetic Lab. working at molecular level and students and physician should be trained so that they can impart genetic counseling to the patients at primary and secondary healthcare centres. Private practitioners and consultants should also keep the data of chromosomal anomalies so that a precise data can be obtained. The pre natal diagnosis of these syndromes should be encouraged there by reducing the morbidity and mortality of these syndromes.

CONCLUSIONS

Chromosomal abnormalities are prevalent in the OPD Patients. At our hospital although the frequency of Down syndrome is 0.0378, Turner syndrome is 0.0246, in Kleinfelter syndrome 0.0070 and in Edward syndrome is 0.0026 per 1000 patients. This rate is still low as compared to published data of the other hospitals, which may be due to low referral from of our region. Addiction to tobacco, passive smokers and other tobacco products may be one of the causes of aneuploidies. The medical staff at primary and secondary health care centres should be trained in diagnosing the suspected genetic disorders; prenatal diagnosis of above syndromes will reduce the morbidity and mortality. More work is required at tertiary care hospitals to get more relevant data.

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