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IMPACT OF CONSANGUINEOUS MARRIAGES ON GENETIC DISORDERS AS REVEALED IN KARYOTYPING STUDIES IN STUDENTS OF SPECIAL SCHOOLS OF ISLAMABAD, PAKISTAN

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ABSTRACT

Consanguinity is most common phenomenon in Muslim countries, especially in Pakistan, causing physical and mental disabilities in children. The aim of this study was to elaborate the consanguinity effects in Pakistani population by karyotyping method. Blood samples from consented (n = 68) mentally retarded individuals were collected from handicapped children of special schools in Islamabad. The samples were processed and stained for karyotype analysis and images were obtained by high-quality microscope. Karyotyping of 68 mentally retarded individuals were performed to determine the genetic causes. Among the total karyotypes, 24 (35.28 %) were found abnormal, showing chromosomal aberrations. Out of these 24 abnormal karyotypes, 12 cases (17.64%) of trisomy were found in the form of Down's syndrome being the most frequent. In rest of the population, 5 children (7.35%) were monosomic (Turner syndrome), 5 (7.35%) were mosaic turner syndrome, only 1(1.47%) from each of Klinefelter and Cri du Chat syndrome. Finally, ratio of handicapped children having consanguine and non-consanguine origin was found to be 61.76 and 38.24%, respectively. Our findings demonstrate that consanguinity is contributing towards the origin of abnormalities in Pakistani population more than the cross marriages. By creating a sense of understanding in the people about the drawbacks of consanguinity, severe genetic abnormalities can be avoided.

Key words: cytogenetic; consanguinity; syndromes; chromosomal aberrations; mental retardations.

INTRODUCTION

It is a tradition in many countries around the world to marry with close family members leading to high rates of genetic disorders. Cousin marriages in Pakistan were reported more than 60% of the population in 2014. It has been observed that consanguineous marriages account for one billion people live with such population communities (Bittles *et al.*, 2010; Modell and Darr, 2002). Cousin marriages are common in different regions and communities of West Asia, Middle East and North Africa, where the ratio of intra-familial marriages account for approximately 20–50% (Bittles, 2011; Tadmouri *et al*., 2009). Primary health care providers may face challenges to provide counseling to cousin couples in communities where consanguinity rate is high. It is evidenced that countries and communities with high cousin marriages, the consanguineous couples to be found curious to find the solutions of expected abnormalities in siblings (Bennett *et al*., 1999).

The dominance and prevalence of both the first cousin marriage and consanguinity rates differ within same populations and between other communities, depending on religion, society back ground, culture and topography. Cousin marriages are also common in countries, such as Pakistan, Turkey, North Africa, Lebanon, residents in Europe, North America and Australia (Hamamy *et al*., 2011; Schulpen *et al*., 2006). Studies revealed that communities with high consanguinity rates may have large family size and can induce autosomal recessive expressions. Now the society trend is changing, those couples having cousin marriages are planning to have a baby seeking preconception genetic advice for fear of consequences of consanguinity on their children.

Mental retardation is one the most frequent condition in handicaps children with a major impact on the life of persons, their family and society by employing the substantial demands on the society and the health systems (Pollak, 1993; Swaiman, 1994). It is common anomaly which imposes different societal burdens like medical, psychological and social. About 3% of the population is affected but the exact pathogenesis is still poorly understood (Birch *et al*., 1970; Curry *et al*., 1997). It is a challenging task for medical specialist to establish the etiological diagnosis of patients, as the range of possible real disorders is enormous and additional investigations is extensive. Pakistani researchers identified 30 new genes in 2016 with children who were mentally retarded due to cousin marriages. The complete diagnostic cost of a child with mental retardation is still a major load on many healthcare systems. Therefore, every aspect of diagnosis and investigation is useful.

Previous studies showed that genetic disorders are twice more common in consangual marriages as compared to non-consanguine couples in Pakistan (Ansari *et al*., 2004). It has been observed that many factors like social norms, predominate the cousin marriages in the country (Hussain, 1999). Other reasons include the strengthening of family relationship and monetary benefits (Hussain and Bittles, 1998). Consanguinity has been reported previously about 60% out of which 80% observed in the form of cousin marriages (Hoodfar and Teebi, 1996).

Considering the population of Pakistan and high fertility rate with cultural background of cousin marriages, the problem of inherited disorders and congenital malfunctions may be significant. Present study is, therefore, designed to evaluate and assess the impact of cousin marriages in Pakistani population by studying the karyotypes of mentally retarded children of special schools.

MATERIALS AND METHODS

Blood sampling:

Sixty eight (68) mentally handicapped children were selected for chromosomal analysis from National Institute for handicap children G-8/2 and Rehabilitation Center for Mentally Retarded Children, H-8/4, Islamabad. Ethical committee of Quaid –I Azam University Islamabad approved the study. Consent from children and their parents were taken to participate in this study. Blood samples were collected in 5 ml tube (2-4 mL) containing sodium heparin as anti-coagulant (Vantage). The tube was inverted several times to prevent the clotting. The samples were stored at 4 ºC.

Culturing

From each sample 0.5-1 mL of blood was added in 15mL falcon tubes (SANAKA), containing 5mL RPM1 Culture media (GIBCO). A mixture of antibiotic streptomycin and penicillin (GIBCO) 0.05-0.1mL was added to each sample. Then 0.2mL of Fetal Bovine Serum (GIBCO) was added to each sample and finally 0.1- 0.2mL of phytohemagglutinin (M form) (PHA) (GIBCO) was added to each tube. PHA acted as a mitogen and brought about the cell division of lymphocytes which otherwise were in interphase state. Each tube was labeled with patient number carefully. The samples were kept in an incubator at 37ºC for 72 hours. Culture was gently mixed 2-3 times every day during incubation. At 70^{th} hour of culturing, 0.075 mL of colcemid was added to arrest the cell division. Aseptic conditions were maintained during the whole process of culturing.

Harvesting

After1-2 hours of treatment with colcemid, cells were harvested using the following technique. First of all, 6-8mL of pre-warmed 0.56% hypotonic solution of KCl (BDH) was added to each tube and kept it in incubator for 10 minutes. Hypotonic treatment allowed the swelling of the cells, which helped in the spreading of chromosomes. The tubes having cell cultures were centrifuged at 150xg and the supernatant was decanted. Then 7mL of fixative was added to each tube and cooled in refrigerator for 10 minutes to fix the cells. Fixed cell suspension was centrifuged for 7 minutes at 1000rpm. Supernatant was removed. Added the fixative and centrifuged again to break the pellet. Sometimes to break up the pellet completely a Pasteur pipet were used to bubble the air through the suspension. Finally, the transparent suspension was obtained. Culture is now ready for slide making best results obtained when the slides were made on the same day as soon as the harvesting was completed.

Giemsa banding

Following strategy was opted for Giemsa staining. Phosphate-buffer saline (PBS, PH 7.0) was dissolved in 8.0 g of NaCl with 0.2 g of KCl plus 0.92 g of anhydrous sodium phosphate dibasic Na_2HPO_4 and 0.2g of anhydrous potassium phosphate monobasic (KH_2PO_4), 0.05% trypsin solution in one litre distilled water. Dissolved 35 mg of trypsin 1:250 (Difco) in 70 mL of PBS. The solution is stable for approximately one day. Giemsa stain. 2.5 mL of giemsa (Gurr's) was dissolved in 45mL of phosphate buffer (PH 6.8).working giemsa stain, 2-3 mL giemsa stock solution was mixed with 5-6 mL leishman stain, 20 mL buffer and 25 mL distilled water, and put into a coplin jar. A solution was prepared with 50ml Hanks Balanced Salt Solution (HBSS) and one mL Fetal Bovine Serum was mixed in another coplin jar (One tablet of HBSS in one litre of distilled water).

Karyotyping

Computerized image analysis system having software (Applied imaging MacProbe Version 4.2) was used. Karyotyping was easier, less time consuming and more accurate. Automated photography is another great advantage of computerized image analysis system; a high-quality laser printer is an integral part of the system and produces

prints of photographic quality. So this system simply captures photographs and prints. So analysis was performed directly at the Zeiss microscope (Gosden *et al*., 1992).

Statistical analysis

SPSS software (Version 17, IL) was used, numerical values were categories in pie chart by using Microsoft excel, the percentages are the numerical variables which are noted in different forms of syndromes/abnormal karyotypes.

RESULTS

Results obtained from analyzed data showed that parents of mentally retarded children having cousin marriages show significantly higher results towards abnormality of children as shown in Fig.1. Equal numbers of male and female students were selected in this research project. So, females (n=13) and male (n=11) were defected with abnormal karyotypes. In this study 61.76% marriages were consanguine and 38.24% parents were non-consanguine.

The figure clearly depicts the disadvantages of consanguinity in Pakistani population. Ratio of mentally retarded children resulting from Cousin Marriages is significantly high (Fig. 1.).

In this study four different types of syndromes are noted; 35.28% showed abnormal karyotype. The percentage of Down syndrome children (17.64%) is found high and the percentage of Turner syndrome abnormality is (14.70%), klinefelter syndrome is (1.47 %) and Cri du chat is (1.47%) as shown in Fig.1.

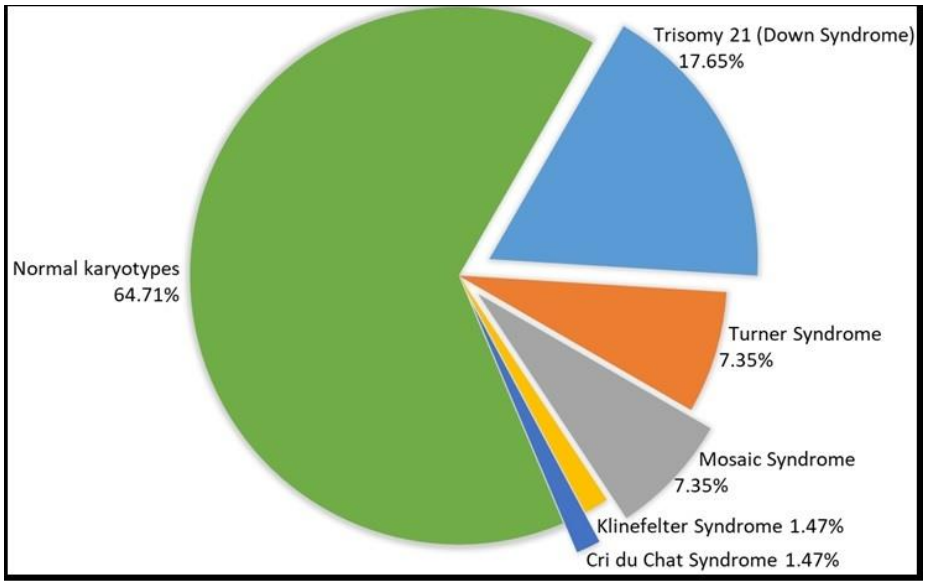

Fig. 1. The distribution of numerical aberrations found in the children during the study. The figure clearly shows the high rate of trisomy in abnormal children. Klinefelter syndrome and turner syndrome's karyotypes are shown in Fig.2 (A & B).

DISCUSSION

Keeping in mind the genetic disorders induced in children resulting from consanguineous marriages in the conservative societies all over the world, present studies were conducted to examine the different types of cytogenetic disorders and their frequencies were compared among the mentally retarded groups of students. It also emphasizes the importance of precise investigation in confirming the chromosomal abnormalities which provide the basis for proper genetic counseling in Pakistani communities. In this research study, family history of mentally retarded children was taken from their parents. After compiling the data, it was keenly observed that in our population cousin marriages are also one of the major causes of mental retardation. In this study, 61.76% cases were consanguine and 38.24% were non-consanguine parents. In consanguine parents, the risk of recurrence of abnormal child is very high. As repeated cousin marriages in family ultimately leads to homozygosity which in turn result in congenital diseases like mental retardation (Hanan *et al*., 2007; Alwan *et al.,* 1997).

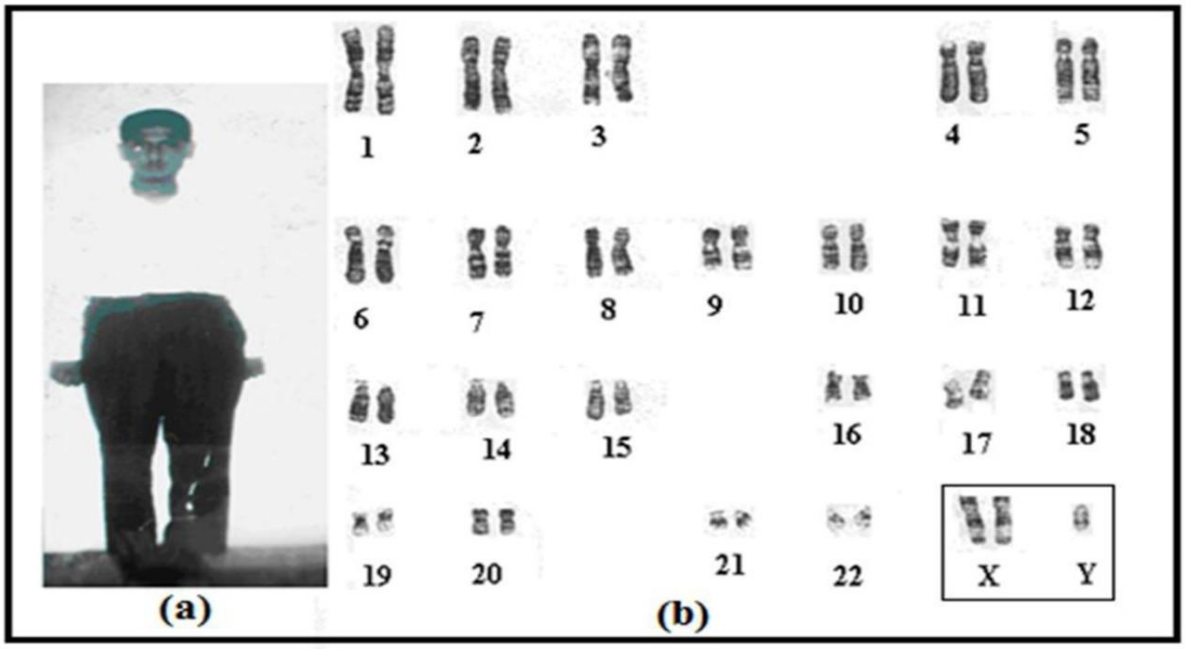

Fig. 2 (A) **Klinefelter syndrome** (a) Male showing Klinefelter's Syndrome Karyotype (b) Chromosome analysis of the peripheral blood culture reveals two X and a Y chromosome as shown in small square.

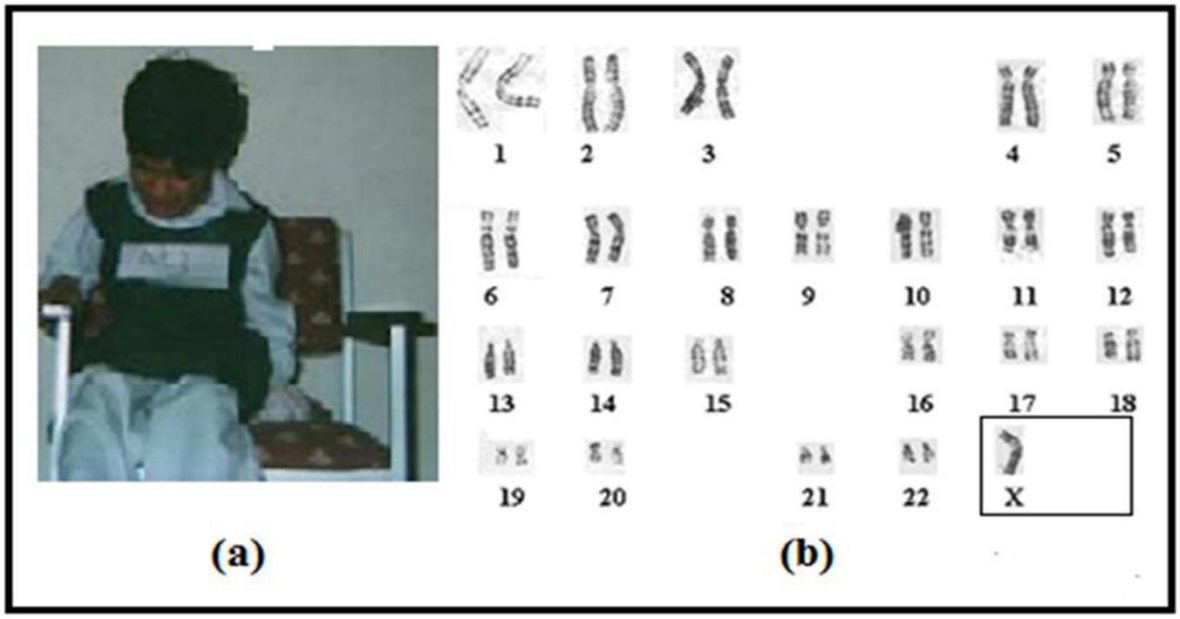

Fig 2. (B) **Turner syndrome** (a) Female showing Turner Syndrome Karyotype (b) Chromosome analysis of the peripheral blood culture reveals with one missing Y chromosome as shown in small square.

In our work, we found 35.28% abnormal cases, whereas a related rate 40% has been reported among 120 patients (Kenue *et al*., 1995). The frequency of chromosomal anomalies are found higher in other studies (15.03%), (13.3%), (17.6%), (32.2%) (Fryns *et al*., 1986; Laxova *et al*., 1977). In these findings, frequencies may differ due to methodology and patients' inclusion criteria.

Down syndrome (DS) due to highest incidence at birth always gained a special interest in the human genetics (Mueller and Young, 1996). 50 % patients in our study were found down syndromic and showed abnormal karyotypes. These results are in align with the studies of (Al-Awadi *et al*., 1985; Kenue *et al*., 1995). In our study we have found 91.66% trisomy 21 cases (Down Syndrome). These values are concomitant with the other related studies ranged from 84.6% to 95% (Gardner and Sutherland, 1996). The rate of regular trisomy 21 in our study have

91.66% value which is higher than 90% (Stoll, 1990 and Cassiman, 1975). However, some frequencies are less than 90% as investigated by (Jacobs, 1978: Jyothy *et al*., 2000). Mosaicism in down syndrome is also reported which vary between 0% to 4.6% (Speed *et al*., 1978; Jacobs *et al*., 1978). Only 1.47 % patients had mosaic syndrome that showed somatic mosaicism karyotype as (46, XX/47, XX+21). All 12 cases of Down syndrome children have ages more than 5 years. Out of 12 DS patients 4 females were observed, but it is difficult to draw any gender base consequence upon small size of sampling and is not suggestive of a higher incidence of males than females with DS in our region.

Turner syndrome patients had a classical 45X karyotype. Most of the patients with phenotypic characteristic such as short stature, web neck, low set ears, low posterior hair line, gonadal dysgenesis and Turner stigmata (Kleczkowska *et al*., 1990). Present findings describe that 10 students were affected with turner syndrome. Out of these, five were mosaic and others had monosomy i.e. 45, X/46, XY or 45, X/46, XX, the reason behind this is the loss by non-disjunction of the Y chromosome after normal disomic fertilization (Lorda-Sanchez *et al*., 1992; Robinson *et al*., 1995).

In this study, out of 24 abnormal cases, five cases were reported as mosaic turner syndrome (20.83%). Mosaic condition in Tuner syndrome is considered as the second number chromosomal constitution (Thomson *et al*., 1991). Out of these cases, one patient showed some clinical characteristics like motor delay, microcephaly and facial dysmorphism which carry a deletion on chromosome 5 (5p-) considered as "cri-du-chat"syndrome.

Klinefelter syndrome is the second most frequent sexual abnormality and shows the typical, distinct phenotype like small testes and gynecomastia, which appears in only 1/3 of the patient (Jorde *et al*., 1996). It has been hypothesized that phenotypic differences in individuals with XXY are primarily related to the presence of the additional X chromosome (Geschwind *et al*., 2000). Klinefelter syndrome (47, XXY) is the set of symptoms which appears in the form of two or more X chromosomes in males. Primary features include infertility and small testicles and often symptoms may be subtle and many people do not realize they are affected.

Cousin marriages are also linked with deafness in different castes, the highest percentage of congenital deafness was found in the Rajput families (Ullah, 2017). These marriages are common in our country and people being unaware of its magnitudes. Therefore, measures should be taken for determining the risk of recurrence, clinical treatment and genetic counseling at community as well as government level.

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DECLARATION

All authors declare, they have no competing interests

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AUTHOR'S CONTRIBUTION: MH: Designed study and performed experiments. KA: did data collection. JI: Help in writing the manuscript.

REFERENCES

Al-Awadi, S.A., A.S. Teebi, T.I. Farag, K.K. Naguib, M.Y. El-Khalifa, S.A. Al-Othman and T.S. Sundareshan (1985). A five-year experience with autosomal abnormalities in Kuwait. *Journal of Kuwait Medical Association*, 19: 269-275.

Alwan, A.D., B. Modell, A.H. Bittles, A. Czeilel and H. Hamamy (1997). *Community control of genetic and congenital disorders*. World Health Organisation. Office for the Eastern Mediterranean.

Ansari, M.A., H. Ismail and I.A. Muhammad (2004). Consanguinity as a cause of deafness and hearing impairment in Karachi. *Pakistan J Otolaryngol*,, 20: 22-6.

Bennett, R.L., L. Hudgins, C.O. Smith and A.G. Motulsky (1999). Inconsistencies in genetic counseling and screening for consanguineous couples and their offspring: the need for practice guidelines. *Genetics in Medicine*, 1(6): 286-292.

Birch, H., A. Richardson, B. D. Horobin and G. Illsley (1970). *Mental Subnormality in the Community: A Clinical and Epidemiologic Study*. Baltimore, Williams & Wilkins Co.

Bittles, A. (2011). The global prevalence of consanguinity. http://www. Consang.net accessed.10-12.

Bittles, A.H. and M. L. Black (2010). Consanguinity, human evolution, and complex diseases. *Proceedings of the National Academy of Sciences-USA*, 107(suppl 1): 1779-1786.

Cassiman, J.J., J.P. Fryns, J. De Roover and H.Van den Berghe (1975). Sex chromatin and cytogenetic survey of 10417 adult males and 357 children institutionalized in Belgian institutions for mentally retarded patients. *Humangenetik*, 28(1): 43-48.

Curry, C.J., R.E. Stevenson, D. Aughton, J. Byrne, J.C. Carey, S. Cassidy, C. Cunniff, Jr, J.M. Graham M.C. Jones, M.N. Kaback and J. Moeschler (1997). Evaluation of mental retardation: recommendations of a consensus conference. *American journal of medical genetics*, 72(4): 468-477.

Fryns, J.P., A. Kleczkowska, A. Dereymaeker, M. Hoefnagels, G. Heremans, J. Marien and H.V.D. Berghe (1986). A genetic-diagnostic survey in an institutionalized population of 173 severely mentally retarded patients. *Clinical genetics*, 30(4): 315-323.

Gardner, R.J. and G.R. Sutherland (1996). *Elements of medical cytogenetics. Chromosomal Abnormalities and Genetic Counseling*. Oxford University Press, Oxford,6-9.

Geschwind, J.F.H., D. Artemov, S. Abraham, S. Omdal, M.S. Huncharek, C. McGee, A. Arepally, D. Lambert, A.C. Venbrux and G.B. Lund (2000). Chemoembolization of liver tumor in a rabbit model: assessment of tumor cell death with diffusion-weighted MR imaging and histologic analysis. *Journal of Vascular and Interventional Radiology*, 11(10):1245-1255.

Gosden, C., C. Davidson and M. Robertson (1992). Lymphocyte culture. In: *Human Cytogenetics: A Practical Approach* (Rooney DE, Czepulkowski BH, editors). IRL Press Ltd. Oxford, UK:31-54.

Hamamy, H., S.E. Antonarakis, L.L. Cavalli-Sforza, S. Temtamy, G. Romeo, L.P. Ten Kate, R.L. Bennett, A. Shaw, A. Megarbane, C. van Duijn and H, Bathija (2011). Consanguineous marriages, pearls and perils: Geneva international consanguinity workshop report. *Genetics in Medicine*, 13(9): 841-847.

Hanan A., D. Hamamy T. Amira D. Masri M. Azmy D. Al Hadidy M. Kamel and D. Ajlouni (2007). Consangunity and genetic disorders. *Saudi Med J.*, 28: 1015-1017.

Hoodfar, E. and A. S. Teebi (1996). Genetic referrals of Middle Eastern origin in a western city: inbreeding and disease profile. *Journal of medical genetics*, 33(3): 212-215.

Hussain, R (1999). Community perceptions of reasons for preference for consanguineous marriages in Pakistan. *Journal of biosocial science*, 31(4): 449-461.

Hussain, R. and A.H. Bittles (1998). The prevalence and demographic characteristics of consanguineous marriages in Pakistan. *Journal of biosocial science*, 30(2):261-275.

Jacobs, P.A., J.S. Matsuura, M. Mayer and I.M. Newlands (1978). A cytogenetic survey of an institution for the mentally retarded: I. Chromosome abnormalities. *Clinical genetics*, 13(1): 37-60.

Jyothy, A., K.S.D. Kumar, G.M. Rao and V. Babu (2000). Cytogenetic studies of 1001 Down syndrome cases from Andhra Pradesh, India. *Indian Journal of Medical Research*, 111: 133.

Kenue, R.K., A.G. Raj, P.F. Harris and M.S. El-Bualy (1995). Cytogenetic analysis of children suspected of chromosomal abnormalities. *Journal of tropical pediatrics*, 41(2): 77-80.

Kleczkowska, A., E. Dmoch, E. Kubien and J.P. Fryns (1990). Cytogenetic findings in a consecutive series of 478 patients with Turner syndrome. The Leuven experience 1965-1989. *Genetic counseling* (Geneva, Switzerland), 1(3-4): 227-233.

Laxova, R., M.A.C. Ridler, M. Bowen-Bravery and J.M. Opitz (1977). An etiological survey of the severely retarded Hertfordshire children who were born between January 1, 1965 and December 31, 1967. *American Journal of Medical Genetics*, 1(1): 75-86.

Lorda-Sanchez, I., F. Binkert, M. Maechler and A. Schinzel (1992). Molecular study of 45, X conceptuses: correlation with clinical findings. *American journal of medical genetics*, 42(4): 487-490.

McInnes, R.R. and H.F. Willard (1991). *Thompson & Thompson Genetics in Medicine*. Philadelphia: WB Saunders Company, 231-246. …… Please find if cited?

Modell, B. and A. Darr (2002). Genetic counselling and customary consanguineous marriage. *Nature Reviews Genetics*, 3(3): 225-229.

Mueller, R.F. and I.D. Young (1996). Genetics and congenital abnormalities. In: *Emery's Elements of Medical Genetics*. 9th Edition. Edinburgh: Churchill Livingstone, pp.193-204.

Pollak, M. (1993). Handicapped children. In: *Text book of Developmental Pediatrics* (Pollak M, ed.). Edinburgh, United Kingdom, Churchill Livingstone, 459-504.

Robinson, W.P., F. Binkert, F. Bernasconi, I Lorda-Sanchez, E.A Werder and A.A. Schinzel, (1995). Molecular studies of chromosomal mosaicism: relative frequency of chromosome gain or loss and possible role of cell selection. *American journal of human genetics*, 56(2): 444-451.

Schulpen, T.W., J.C. Van Wieringen, P. J. Van Brummen, J.M. Van Riel, F.A. Beemer, P. Westers and J. Huber (2006). Infant mortality, ethnicity, and genetically determined disorders in The Netherlands. *The European Journal of Public Health*, 16(3): 290-293.

Speed, R.M., A.W. Johnston and H.J. Evans (1976). Chromosome survey of total population of mentally subnormal in North-East of Scotland. *Journal of medical genetics*, 13(4): 295-306.

Stoll, C., Y. Alembik, B. Dott and M.P. Roth (1990). Epidemiology of Down syndrome in 118,265 consecutive births. *American Journal of Medical Genetics*, 37(S7): 79-83.

Swaiman, K. F (1994). Intellectual disability. In: *Pediatric Neurology: Principles and Practice* (Swaiman KF, ed). St. Louis, Missouri: Mosby Yr Book, 133-146.

Tadmouri, Ghazi O., Pratibha Nair, Tasneem Obeid, Mahmoud T. Al Ali, Najib Al Khaja and Hanan A. Hamamy (2009). Consanguinity and reproductive health among Arabs. *Reproductive health*, 6(1): 17.

Ullah, S., M. Aslam khan, A. Ali and M. Idrees (2017). Causes of deafness in the Punjab region of Pakistan and the role of consanguinity. *Public health*, 145: 93.

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