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Down Syndrome Survey in South Indian Population- Understanding Inheritance, Perceptions, Interventions and Diagnosis

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ABSTRACT Down syndrome (DS) has been prevalent worldwide, for centuries now. Despite the fact that tremendous research has been done on DS ever since the early 1950s, most results obtained, are on the basis of etiological and demographic factors and predominantly of the western data. Every year in India, >30,000 children are born with DS. This survey has been done keeping the Indian population in mind and to analyze the outlook of parents having children with DS, understand the comorbidities and their management. The study was conceptualized to create an exhaustive and comprehensive questionnaire to study the pattern of inheritance of Trisomy 21, analyze influence and correlation of advanced maternal age, sex ratio, order of birth, hypothyroidism, common comorbidities, abortions, Attention deficit hyperactivity disorder (ADHD) and sleeping difficulties in individuals diagnosed with DS. 50 family triads were interviewed. The results showed that incidence of DS was more in males compared to females. The analysis revealed that mean maternal age of 25-28 years showed increased incidence of DS. 2.5 percent showed severe ID and 27.5 percent had severe ADHD symptoms, while 10-13 percent showed mild to moderate ADHD. The most and least prevalent comorbidity seen was the presence of heart disease (45%) and hearing impairment (10%) respectively. It was found that about 40 percent of parents strongly agreed to the idea that Genetic Counseling (GC) is helpful and wanted to reinforce it to others who find it difficult to cope up with their DS child.

INTRODUCTION

Down Syndrome is a form of aneuploidy in humans affecting all races and economic levels. It is observed as the most prevalent genetic condition worldwide (Megarbane et al. 2009). It is to be noted that DS is one of the major reasons for mild to moderate intellectual disability not just in India, but globally. DS children find it difficult to concentrate and have lowered focus ability and get confused easily by environmental factors (Bull 2011). It is to be noted that children with DS exhibit positive personality characters such a friendliness and tenderness (Makhov and Medvedev 2020). IQ is classified into severe, mild and moderate, where the IQ for each stage stays in a particular range.

The inheritance of DS has not yet been completely understood. The medical termination of pregnancy after prenatal diagnosis has not increased while the incidence rate of DS has not decreased in the last decade (Baikie et al. 1959).

Genetic Counseling (GC) is defined as classification and diagnosis of a genetic disease, where the prime drive is identifying carriers; to counsel them regarding the risks associated with having

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affected children. GC also means to identify a severe genetic disease even before the symptoms arise, so that the quality of life can be improved. If proper awareness is given to these parents on the importance of GC, their perceptions might change and thus help the DS individual and their family to cope better. This will also make them understand that DS affected individuals can lead a close to normal lifestyle.

Since the lifespan of people with DS has increased over time owing to better medical facilities, getting a much deeper insight on DS is important and necessary in order to identify an intervention or management method to improve the lifestyle of DS individuals. This paper is based on the information collected from 50 families by utilizing a questionnaire specially created to analyse various psychopathological and genetic factors pertaining to the respective individuals with DS. Surveying of the questionnaire enabled us to collect valuable, authentic information regarding the condition of the participant.

Objectives

The objectives of the present research are:

Objective 1: To create an exhaustive and comprehensive questionnaire and test the same in 50 family triads belonging to the South Indian Population.

Objective 2: To analyse and correlate the genetics and expression of Trisomy 21 with advanced maternal age, sex ratio, order of birth, hypothyroidism, common comorbidities, abortions, ADHD, sleeping difficulties in individuals diagnosed with DS.

Etiology

Chromosome 21 has 47 million nucleotides that represent ~1.7 percent of the human genome. It has a size of approximately 60Mb with 400 genes present on chromosome 2 (Hattori et al. 2000).

There are various hypotheses that are related to the genetics of DS. The most widely accepted hypothesis signifies that gene proliferation is caused due to increase in the total number of genes or increase in dose of chromosome 21 and that there might be a relationship between other genes in the occurrence of DS. The amplified development instability theory which states that genetic discrepancy caused by a number of trisomic genes has an impact on other gene regulatory systems is another well-known hypothesis (Asim et al. 2015).

Critical region hypothesis enunciates that chromosomal regions such as Down Syndrome Critical Regions (DSCR) are responsible for the partial trisomy in Hsa21. Several clinical features of DS are caused due to the expression of DSCR in the chromosome locus 21q21.22 (Bittles and Glasson 2004; Asim et al. 2015). It has been concluded from several studies that the clinical features of DS is not an effect of a single critical region but multiple and several critical regions (Bittles and Glasson 2004).

Epidemiology

DS affects 1 in 700 neonates worldwide. Trisomy, the most common of all DS types accounts for 95 percent, Robertsonian Translocation for 3 percent and Mosaic for 2 percent of total DS population (Roper and Reeves 2006). In a survey of 94,910 newborns, DS births in Mumbai, Delhi, and Baroda were 1 per 1150 newborns (Verma 2000).

Trisomy 21(Ts21) consists of the following comorbidities- seizures, dysfunctional thyroid, Alzheimer's disease, diabetes, and dementia. It is one of the major causes of congenital heart disease, acute megakaryoblastic leukemia (AML) and developmental cognitive deficits (Bull 2011). From the literature the researchers find that 76 percent of DS children suffer from hearing loss, ~65 percent with sleep disorders and ear infection, 62 percent eye disease, ~25 percent psychiatric disorders, and more than 50 percent from heart diseases (Myers and Pueschel 1991; Roizen 1996). DS causes intellectual disability in nearly 17 percent of the population suffering from ID and associated disorders across the world. DS is also shown to be associated with ADHD (Roizen 1996).

Pathophysiology

Free Trisomy 21

It is caused by a nondisjunction that occurs in cell division. This results in the embryo having three copies of chromosome 21, that is, 47 chromosomes in total. Prior to or during fertilisation, the sperm or the egg fails to separate leading to a sporadic occurrence of trisomy 21. This 21 is now

copied throughout the human body cells. Maternal meiosis I accounts for > 75 percent of nondisjunction, maternal meiosis II errors account for >20 percent (Eggermann and Morris 2011). Analysis of chromosome heteromorphisms and DNA markers of patients and their parents revealed that the nondisjunction occurred more often during conception in females than in males. Investigations have revealed that the extra 21 mainly originates from meiosis errors in women older than 35 years of age.

Tetrasomy 21

This is a hyperploidy (four copies) of the 21st chromosome (Eggermann and Morris 2011). This condition is lethal in the developing stage. By literature, the only case identified was by Gardner and Sutherland in the year 2004.

Mosaicism

When an individual has more than one unambiguous cell line originating from the same zygote, then it is a case of Mosaicism. Mosaic individuals have trisomic as well as euploid cells (Clarke et al. 1961). Presence of Mosaicism is not easily identifiable in Ultrasound detection (Bornstein et al. 2009). Around 30 spreads of metaphase have to be analysed to rule out mosaicism, according to the American College of Medical Genetics (Shin et al. 2010).

Mosaicism involving DS arises via (1) Non-recombinant errors; (2) Mitotic errors; (3) Uniparental disomy. Mosaic individuals having more trisomy 21 cells than individuals with lower trisomy 21 cell composition have more prominent clinical features. Mosaic kids develop gross motor skills faster than those without mosaicism. It is little known about the fertility in mosaic DS individuals, although they turn out to be more social (Papavassiliou et al. 2015).

Robertsonian Translocation (RT)

A translocation involving a segmental exchange of chromosomes was 1st reported in DS. Sir Polani described the 1st translocation involving chromosomes 13-15/21 (Scriven et al. 2001). When chromosome 21 attaches with an acrocentric chromosome (13,14,15, 21 and 22), it accounts for Robertsonian Translocation (75% *de-novo* and 25% familial).The translocation partner frequency varies, which leads to non-fulfillment in meiosis I. Meiosis in germ cells with balanced translocations might result in infertility. Translocation involving chromosomes 21/21 have uncommon repositioning (Sergovich et al. 1962)

Usually, the carriers of RT show 46 chromosomes having regions lost in the short arm. The most common RT is (14q21q) having 60 percent, followed by (21q21q) in 35 percent of cases while the other translocations are less than 5 percent. Balanced translocations, including Robertsonian and reciprocal translocations are benign (Hubert et al. 1962). The chance of inheritance of this translocation depends on the gender of the parent who is the carrier. Close relatives of translocation DS have greater chances of being carriers (Flinter et al. 2001).

Reciprocal translocations involve interchange of euchromatic regions between Chromosome 21 with other autosomes. Most of the reciprocal translocations are unique. Duplication happens *de-novo.* When there is a Paracentric inversion in the 21st chromosome, there is a chance of duplication. The carrier might have a mild impairment if the segment duplicated is smaller. When homologous chromosomes are unequally paired, duplication of carrier's cells occurs (Eggermann and Morris 2011).

Clinical Features

Congenital Heart Defects (CHD)

CHD is the leading cause of death in DS. Atrioventricular septal defect (AVSD) is the most common, constituting 40 percent of CHD in DS due to the mutation of the non-Hsa21 *CRELD1* gene (Antonarakis et al. 2004). Tetralogy of Fallot constitutes 6 percent, Ventricular septal defect (VSD), constitute 32 percent of DS, secundum atrial defect (10%), and isolated PDA (4%). According to literature, VSD is found to be more common in Asian DS kids while secundum type is common in Latin America (Wiseman et al. 2009).

Gastrointestinal (GI) Tract Abnormalities

The commonly associated GIT problems are annular pancreas, Hirschsprung disease, imper-

forate anus, stenosis, chronic constipation, intermittent diarrhea, gastroesophageal reflux (GERD), and celiac disease (Wallace 2007).

Hematologic Disorders

Hematologic disorders like neutrophilia, polycythemia and thrombocytopenia are commonly associated with DS (Hord et al.1995; Henry et al. 2007). Myeloproliferative disorder is often seen in 3-month-old or younger babies (Miller et al. 1983). Myelopoiesis is found in10 percent of patients and found to cause spontaneous abortion (Zipursky et al. 1997; Kearney et al. 2009). One third of DS individuals suffering from acute lymphoblastic leukemia have been found to be associated with Janus Kinase 2 gene mutation (Kearney et al. 2009). Acute megakaryoblastic leukemia (AMKL) is related to gene *GATA1*, which leads to immature megakaryocytes proliferation (Wechsler et al. 2002).

Endocrinological Disorders

The dysfunctional thyroid gland, particularly hypothyroidism (elevated TSH and normal thyroxine levels), is commonly associated with DS, which can be acquired or congenital. Hyperthyroidism is relatively less common. The delayed skeletal maturation with short stature is due to insulin-like growth factor (Lavard et al. 1994).

Musculoskeletal Disorders

DS children have hypotonia that leads to gross motor skills retardation, dislocation of joints, fractures and malabsorption of vitamin D (Hawli et al. 2009).

Refractive Errors and Visual Abnormalities

Common anomalies in DS kids are ocular and orbital such as retina deformation, cataract, keratoconus, optic nerve anomalies, blepharitis, strabismus, refractive errors, iris anomalies, and glaucoma. Eye check-up is mandatory for DS individuals. Hearing loss involves chronic middle ear effusion, acute otitis media, and eardrum perforation (Merrick and Koslowe 2001).

Screening

Ultrasound Scan

Aneuploidy is a major reason for dismalness and mortality and can significantly affect the guardians and their families. With early diagnosis and screening, we can sensitize the parents about the potential effect of analysis. An ultrasound will help in the visualisation of DS-specific features in fetuses. Though these imaging techniques are widely used for the identification of DS, the chance of false positive or false negative result cannot be rule out. (Thomas 2017).

The routine ultrasound is done at the 18^{th}-22^{th} week of pregnancy and can uncover an assortment of physical attributes that are related to a child being born with DS. The sounds travel via the amniotic fluid and are reflected by uterine tissues. Based on the density of what they contact, the waves bounce back at different speeds. This data is converted into an image of the fetus by a software. Clear image of the fetus is obtained if the structure is dense and hard (Rao et al. 2016). A fetus with DS shows subtle signs, called soft markers.

Non-Invasive Prenatal Test (NIPT)

NIPT is a non-invasive test in which the DNA of the fetus is isolated from maternal blood and analysed for genetic abnormalities. This method is preferred over the first and second trimester tests for DS. In the case of DS, it is positive and an invasive test confirms the result. The result shows negative when the individual is not likely to be affected. About 4 percent of the cases are inconclusive as the amount of fetal DNA present is not accurate.

Markers Associated with DS

During the 2^{nd} trimester, nuchal fold (neck area) thickening of size $\geq$ 6 mm is an important marker to detect the presence of DS. Most foetuses (~88%) with 3 mm nuchal translucency (NT) during the 12^{th} week of gestation are born normal while, 12 percent possess abnormalities (Benacerraf and Frigoletto 1987). NT is known as the physiological marker of DS as it reflects the vascular and fetal lymphatic process in the develop-

ing baby. When increased NT is found in a fetus, the mothers should undergo CVS along with an ultrasound to rule out the chances of abnormalities detection at birth (Berger 1999).

DS has another marker called Pyelectasis that indicates the renal pelvis diameter ($\geq$ 4mm). Renal dilation is common in DS fetuses. Due to low sensitivity, Pyelectasis is a minor marker (Corteville et al. 1992). Missing nasal bone in the fetus during the 1st trimester is a marker of importance, 73 percent Trisomy 21 fetuses have this condition (Cicero et al. 2001). The marker with the lowest sensitivity is the echogenic foci, for identifying DS. It is detected in 15 percent of DS cases (Shanks et al. 2009). A valuable indication is an iliac angle marker which is abnormal (90.32^0) in the embryo for DS. The normal measurement is 63.72^0 (Belics et al. 2011).

1st Trimester Screening (Nuchal Translucency Ultrasound)

This screening involves a combination of ultrasound examination along with serum markers, carried out in the 10th to 14th weeks of development. The motivation behind a screening test is just to recognize a subset from the screened populace to whom the diagnostic test should be considered.

Sometimes fluid gets collected in the neck region of the fetus leading to damages in the pathological pathways. The accumulation of fluid at the back of the neck is NT which can be detected and measured by ultrasound. Ductus venosus flow is another sensitive parameter for first-trimester screening (Carlson and Vora 2017) The BPD/FL proportion and the nuchal skin fold thickness along with the maternal age rate determine the likelihood of DS in a specific baby.

PAPP-A (Pregnancy-associated plasma protein A) and β-hCG (beta-human chorionic gonadotropin) are the serum markers that are used widely. The mother's age, weight, pregnancy history, serum markers, nasal bone condition, race, and NT measurement for risk is estimated. Advantages of this screening comprise of early detection and results, which allows the caregivers to make further decisions of the pregnancy (Raniga et al. 2006).

Quadruple Screening

Mid trimester screening for DS is carried out in the 15th and 22th weeks of gestation. Associated serum markers are hCG, alpha-fetoprotein (AFP), inhibin A, and unconjugated estriol. These markers with the parents' ethnicity, gestational age, presence of diabetes, etc. gives an estimation of occurrence for DS.

Prenatal Cell-Free DNA Screening (cfDNA)

This test involves collecting the maternal blood, from which cell-free DNA (cfDNA) of the fetus is isolated. The cfDNA is basically placental in origin. In contrast to most DNA, found within the nucleus, these sections are free-floating and not inside cells, and so are called cfDNA. During pregnancy, the mother's circulatory system contains a blend of cfDNA that originates from the mother's cells and also the placenta. The placenta in the uterus is a tissue that interfaces the embryo and the mother's blood supply. The DNA in placental cells is identical to the DNA of the fetus. These cells are shed into the mother's circulatory system throghout pregnancy. Dissecting cfDNA from the placenta gives a chance to early recognition of genetic conditions without any harm to the developing embryo. There must be sufficient fetal cfDNA in the mother's circulatory system to distinguish the fetal chromosome. The extent of cfDNA in maternal blood that originates from the placenta is known as the fetal fraction. There are two outcomes, increased risk or low-risk, presence or absence of chromosomal abnormality, making this blood test most sensitive for DS detection (Gil et al. 2015).

Maternal Blood Tests

Negative screening results imply the chance of DS is low, yet they don't ensure the absence of birth anomalies. Positive screening results imply the odds of having a baby with DS are higher than ordinary, thus follow-up testing will be recommended.

Diagnostic Testing

Karyotype

A karyotype is a genetic test that is used widely to identify the number of chromosomes, size and shape of the chromosome present in an organism. Karyotyping is considered the gold stan-

dard for the diagnosis of DS. Under ordinary conditions, there are 46 chromosomes sorted in 23 sets. On account of DS, there is an additional chromosome in the 21st spot, which means there are three copies of this specific chromosome. The sensitivity rate for DS in karyotyping is 99.5 percent.

Chorionic Villus Sampling (CVS)

An important tool for prenatal diagnosis during the first 14 weeks of gestation is CVS. Couples at risk of having a baby with a chromosomal disorder are suggested for chorionic villus examination. The CVS samples obtained help in chromosome analysis, enzyme determinations, and DNA analysis. CVS is done at 10 to 14 gestational weeks (early diagnosis) as the villi starts to vanish after this period. The obtained villus sample contains a large number of cells that are actively dividing, thus a karyotype is obtained within 5 to 24 hours. This early diagnosis of CVS fetal karyotyping (done within 14 weeks) helps parents in making any pregnancy-related choice while decreasing medical complexities. The earlier diagnosis helps with early intervention. The risk of abortion is 1percent (Simoni et al. 1983).

Amniocentesis

Amniocentesis involves withdrawing amniotic liquid from the uterine cavity through a needle, by means of a trans abdominal approach and with continuous monitoring using ultrasound. Various chromosomal, biochemical, atomic and microbial investigations are performed on the amniotic liquid sample. It is performed from four months of pregnancy onwards. The technique has a danger of fetal loss of 0.5 percent when performed in the second trimester when the amniotic layer has combined with the chorion. There is likewise a risk of amniotic liquid spillage, placental discharge, intra amniotic contamination, stomach divider hematoma, and fetal injury. Around 20 mL of amniotic liquid must be collected without defilement by maternal cells. The fetus must be constantly monitored during the process by ultrasound. In Rh-negative patients, seroprophylaxis with anti D immunoglobulin is performed (Shohat et al. 1995).

Cordocentesis

Cordocentesis otherwise called percutaneous umbilical blood testing is the examination of foetal blood obtained from the umbilical cord. A needle is inserted through the mother's abdominal cavity under ultrasound guidance. It is normally performed at 18 weeks of development. The risk of abortion is 1.4 percent. Practice of cordocentesis is relatively uncommon due to the presence of amniocentesis and chorionic villus examining, which represent a lower rate of fetal loss. The risk related are fetal loss, cord hematoma, feto-maternal loss and other infection (Tongprasert 2007).

Fluroscence In Situ Hybridization (FISH)

A commonly used molecular-cytogenetic tool, FISH helps in DNA and chromosomal examinations. FISH comprises a hybridizing DNA probe, to label the chromosomes either directly or indirectly. In direct marking, nucleotides that are fluorescent are utilized, while for indirect it also comprises of other molecules to be taken up by the antibodies .The commonly used tool to detect interphase nuclei is FISH, which uses a probe specific to 21. FISH finds usage in distinguishing hereditary anomalies, irregular chromosome quantity or absence of a part or an entire chromosome. FISH is generally done utilizing the tissue sample obtained from an amniocentesis or CVS test (Bishop 2010).

For the FISH investigation, fluorochromes are utilized to identify certain chromosomes as observed in metasystems. In normal cells, we can detect 2 signals of orange for chromosome 21 under a fluorescence microscope while in case of DS (Trisomy 21), 3 orange signals are visualized. FISH can be performed on uncultured cells, which is an added advantage (Bishop 2010). An advantage of diagnosing mosaics in FISH is higher. Though advantages for FISH are may; one major disadvantage is that it lacks in demonstrating the structure of chromosome 21.

Multiplex Ligation-dependent Probe Amplification (MLPA)

MLPA identified in 2002 by MRC Holland can quantify 40 different sequences of genetic material in one reaction using PCR (semi-quantitative) principle. It is used in the field of genetic diagnosis The low quantity of genomic DNA, high specificity rates, cost-effectiveness, sensitivity, and robust nature make it a favorable tool for aneup-

loidy screening even during late pregnancy (Papageorghiou et al. 2001). The procedure may take up to 2 days (Yurdakul et al. 2010).

Quantitative Fluorescent PCR (QF-PCR)

For diagnosing abnormalities, better than conventional karyotyping, QF-PCR is less expensive, quick and suitable. It helps in detecting contamination of maternal cells, reduces the work pressure in cytogenetic labs and also reduces the anxiety of the parents while waiting for the results, as the entire process takes about half an hour (Badenas et al. 2010).

Microsatellite Typing

Peterson described the Short tandem repeats markers (microsatellites) as molecular markers, which help in the detection for the presence of extra chromosomes. Even though it is a rapid method, it does not allow the detection for balanced rearrangements (Eggermann and Morris 2011).

METHODOLOGY

Questionnaire

The questionnaire consists of 47 questions, divided into 7 exhaustive parts. Part 1 includes personal information of the parents and individuals with DS, such as age, family history of DS, ethnicity, etc. Part 2 comprises details of ultrasound scan, maternal serum screening and other prenatal information relevant. Parts 3, 4 and 5 are designed to assess response to three domains involving child growth, medical history, and physiological characteristics. Part 6 required parents to answer open ended questions. The final part consists of a pedigree history of the family from both the maternal and paternal sides. General comments and experiences were also included.

RESULTS

From the survey conducted, most of the children had classic DS features. In the 50 individuals studied most of them were male and the male: female was quantified to be roughly around 33:17 (Table 1). It was also clear from the survey that more than half of the individuals with DS were second born children and that paved a way to study the association of maternal age with the incidence of DS (Table 2).With gradual increase in the age of the mother there was an increase in the number of children born with DS. The maternal age group between 25-28 showed the most significant number of DS births. The lowest incidence was seen in the maternal age group between 21-24 with only 4 children with DS born according to this survey (Table 3). We also analyzed the proportionality between mother's age and the incidence of abortion. It was inferred that the number of abortions was maximum among women of ages 25- 28 years. It was also noted that no abortions were observed in age groups 21-24 years. Amongst the total number of women surveyed, 2 had the choice of MTP while 2 chose not to terminate the pregnancy (Table 4).

Table 1: Ratio of affected male to female Down syndrome individuals

Male affected ratio	*Female affected ratio*
66%	34%

An analysis of the ratio of male to female affected Down syndrome was done and the results suggested that more males were affected than females, 66: 34. Majority of the children affected were found to be Males (Table 1).

Table 2: Correlation of birth order and expression of Down syndrome

1st born	*2nd born*	*>2*
38%	58%	4%

Order of the affected child was analyzed and our data reflected; 38 percent of individuals were the firstborn, 58 percent were second born and about 4 percent more than the 2nd in order of birth. On analysis, we also found that 6 percent of women who had DS as firstborn, continued with the next pregnancy and delivered an unaffected child. Also, about 3 percent of the mothers who have DS kids as 1st born are currently pregnant (Table 2).

Most mothers who had an abortion were spontaneous. The study population was divided into 6 groups ranging from 0-26+ years. The expression of DS was found to be the maximum in the age group of 0-5 years having a maternal age of 25-28 years. Life expectancy of DS children depends on how early intervention/ therapy is provided. The oldest

Table 3: Incidence of DS in correlation to maternal age

Maternal age groups	*Incidence of DS as number*
Age 17-20	5
Age 21-24	4
Age 25-28	19
Age 29-32	10
Age 33+	12

The analysis was carried out by categorizing the maternal age as a factor into 5 different age groups – ages 17-20 years, 21-24 years, 25-28 years, 29-32 years, 33+ years. Our results showed that the maternal age group between 25-28 years showed an increased probability of birthing kids with DS. This was followed by women of the older age group (33+). The lowest occurrence of DS was found to be among the age group 21-24 years (Table 3).

Table 4: Maternal age Vs. Incidence of abortion

Maternal age	*Abortions*	*Number of Mothers*
Age 17-20	1%	5
Age 21-24	0%	4
Age 25-28	7%	19
Age 29-32	2%	10
Age 33+	5%	12

The relationship between maternal age with abortion rate was evaluated. About 7 percent of the mother aged between 25 and 28 had an abortion and later had a child with DS. The least number of abortions were seen in the age between 21 and 24. A slightly increased rate of abortion was seen in age between 17 and 20 as well as between 29 and 30. (Table 4)

individual having DS in this survey was found to be 38 years old (Table 5).

Table 5: Expression of DS in age groups

Age groups	*Number of children*
Age 0-5 years	25
Age 6-10 years	10
Age 11-15 years	3
Age 16-20 years	5
Age 21-25 years	4
Age 26+	3

In order to study the association between DS children with hypothyroidism and the mother who had thyroid during pregnancy, the maternal age group was divided into 4 groups correlating mothers affected with thyroid during pregnancy and the affected child having hypothyroidism. It was found that the increased maternal age is directly proportional to the children being affected with hypothyroidism. Maternal age group 38-44 years showed the maximum percentage of affected children (60%). The minimum correlation was seen in the maternal age group of 24-30 years. While the maternal age group, 17-23 years showed the absence of hypothyroidism in DS children. Amongst these few mothers also suffered from gestational diabetes and hypertension during pregnancy.

The interdependence of Intellectual Disability (ID) and ADHD was evaluated and out of 50 children analyzed, the researchers found that almost 50 percent of children didn't show any ID or ADHD symptoms. 2.5 percent showed severe ID and 27.5 percent had severe ADHD symptoms. While 10-13 percent showed mild to moderate ADHD, 22-30 percent had ID. Uncomplicated ADHD was common in younger children with DS. However, many school-age children with ADHD were found to have disruptive behavior or OCD traits. Other symptoms such as anxiety or extreme irritability were observed in a few cases. It was found that children aged between 0-20 years and 30+ suffer from ADHD leading to their irrational sleeping patterns. Whereas, the maximum level of ADHD was seen in the age group 21- 30 years. ADHD is commonly found in younger children in the form of lower attention span, impulsive behavior and excessive fidgeting, etc. DS kids show these qualities more than usual children of their age.

The common comorbidities like heart defects, visual impairment, hearing loss, malabsorption, sleeping difficulties, hypothyroidism, GIT problems (diarrhea, vomiting, and constipation) were analyzed in the study population and results are explained. The most common comorbidity seen was the presence of heart disease (45%), followed by sleeping difficulties (40%), malabsorption 37.5 percent and hypothyroidism 32.5%.The least common comorbidity was seen in individuals suffering from visual impairment [17.5% (1 having cataract and 6 children wearing glasses)], followed by hearing impairment [10% (partial hearing loss, ages 2-9 years)] (Table 6).

The main focus with regard to GC was whether the parents agreed or disagreed to the effectiveness of under taking a session. It was found that about 40 percent of parents strongly agreed to the idea that GC is helpful and wanted to reinforce it to others who find it difficult to cope up with their DS child. Around 19 percent of the par-

Table 6: Comorbidities expressed in DS individuals

Comorbidities affecting DS individuals	*Number of children affected*
Heart Disease	45
Visual Impairment	17.5
Hearing Loss	10
Malabsorption	37.5
Sleeping Difficulties	40
Hypothyroidism	32.5
GIT	30

Comorbidity of a DS patient refers to the presence of another medical condition in addition to the DS they already possess. The rates at which some of the common comorbidities that affect DS individuals were studied and tabulated. It was found that about 45 percent of DS individuals were encountered with heart disease which is the most widely seen comorbidity followed by sleeping difficulties at 40 percent. Only 10 percent of the DS individuals faced hearing loss (Table 6).

ents did not take any stand in the working of GC. 11 percent and 4 percent of parents disagreed and strongly disagreed respectively to the potential use of GC (Table 7).

Table 7: Parents view on genetic counseling

Will genetic counseling work ?	*Percentage of people*
Strongly agree	40
Agree	26
Neutral	19
Disagree	11
Strongly disagree	4

Focus was to find out the number of people receptive towards GC. On analysis 40 percent of parents in the study group strongly agreed on the need for GC during pregnancy, 19 percent were neutral to this concept, 26 percent of the parents agreed, 11 percent disagreed and 4 percent strongly disagreed (Table 7).

As explained previously, the three main pathophysiology of chromosome 21 aberrations occur due to Trisomy 21, Mosaicism, and Robertsonian translocation which approximately occur in a ratio of 47:1:2 respectively. According to this survey data Trisomy 21 is responsible for more than 90 percent of the chromosomal 21 aberrations (Table 8).

Table 8: Frequencies of chromosome 21 aberrations

Chromosome 21 aberrations	*Incidence rate*
Trisomy 21	94
Mosaicism	2
Robertsonian Translocation	4

Out of the 50 individuals surveyed, our results showed that 94 percent of children have Free Trisomy 21, 4 percent have RT and 2 percent Mosaics (Table 8)

DISCUSSION

The results of the survey showed that the male sex ratio of affected DS individuals was found to be higher. This is in accordance with the literature studies that depict the syndrome to be predominant in males. It was also concluded that male DS individuals were born to young aged couples whereas a female DS individual was born to older aged couples who are more than 35 years of age. The increase in age of the couple can influence the increase in male sex ratio due to the oocytes aging or the over ripening of the sperms (Verma et al. 1987; Kovaleva 2002). One feature common in most trisomies is an increase in the frequency of trisomy pregnancies with increasing maternal age. Increased maternal age alone isn't an indication of DS despite having a higher probability. The highest occurrence of DS was seen in between the maternal ages of 25-28 years (Verma et al. 1987). There is a remarkable correlation between the expression of DS and the incidence of abortions in women of age groups 25-28 years. This observation needs scientific correlations as studies conducted hitherto explains the incidence of abortions and DS in women of advanced maternal age. A recent study concluded that DS intra uterine fetal loss rate (miscarriage or other mortality factors) rises with increase in maternal age (Cuckle and Morris 2021).

According to Markus Neuhäuser and Sven Krackow, the risk of a DS child to be born depends on the number of siblings present along with the gap in-between them (Neuhäuser and Krackow 2007). The present study showed the percentage of 2nd born children are affected with DS at a higher rate (Verma et al. 1987; Kovaleva 2002).

DS is associated with many dysfunctions in the affected individual. These children have a shorter stature than their peers which is due to deficiencies in growth hormone and IGF-1 as well as zinc (Mazurek and Wyka 2015). Zinc deficiency leads to abnormal growth, diminished immunity and hypothyroidism. Hypothyroidism is due

to the overexpression of DYRK1A gene thus affecting DS individuals (Mazurek and Wyka 2015). Nearly half the children surveyed with DS were affected with congenital heart defect particularly atrioventricular septum, followed by sleeping difficulties (Santoro and Steffensen 2021). Studies have proven that malabsorption, incorrect food options are responsible for inappropriate nutrient intake of the DS individual. The way of feeding also influences the daily activities of a child with DS (Ravel et al. 2020).

In most cases DS individuals who exhibit psychological disorders, such as autism spectrum disorder, OCD (Obsessive Compulsive Disorder) , bipolar disorder, and anxiety issues are given antipsychotic drugs to manage them (Moreau et al. 2021).

DS children have chewing and swallowing difficulties that lead to inadequate and inappropriate nutrition intake, giving rise to constipation. Deficiency of vitamin B and abnormal levels of blood homocysteine reduces intellectual-development in DS (Asim et al. 2015). Early neurostimulation can reduce developmental or social-adaptation deficits in the child (Roizen 1996). Usually, ADHD is followed by sleeping difficulties. By literature, free trisomy is seen in 90 percent individuals having DS. RT occurs in 5 percent of all Trisomy 21 (75% de novo in carrier and 25% familial). The frequency of mosaicism is between 3-5 percent (Eggermann and Morris 2011).

40 percent of the study group parents were positive about GC, while 20 percent were unsure.4 percent who disagreed with the need of GC could be due to ignorance and inability to come out of the social stigma. Technological advancements have enabled GC to play a crucial role in health care of many developed countries. Certain parents perceive that their existence is enriched with their DS kid while few see it as a burden. In general, parents are least prepared and hesitant to accept all the information when the child is born. Thus, proper GC can influence a parent's perceptions towards their child.

A distinctive feature in DS is the variance of the condition. Due to the replication of chromosome 21 throughout the body various comorbidities arise. Motor skill improvement can be attained using occupational therapy. Some of the methods of occupational therapy are using the hands as well as other parts of the body, and assisting the child in dealing with sensory inputs from the surroundings. Enhancing mobility and strength of muscles using physical therapy and also help children to function within operational limitations. Improving communication skills by using speech therapy. Very little muscle tone and many other factors that often result in delayed speech and articulation difficulty affect DS kids. Various strategies are offered by Speech therapists to improve communication and the child's muscle tone. ID in DS individuals range from being mild to moderate. Those who have mild ID can mostly take care of their routine activities and lead a close to normal life, whereas individuals who have moderate ID will require an additional support from a third person to fulfill their everyday needs (Williams et al. 2021).

CONCLUSION

As a consequence for the need to meet the current social and professional demands, most couples delay parenthood. The maternal and paternal age have proportionally increased over the last decade. This has a probable correlation with the increased incidence of DS. The profound need and role of GC has become vital under such clinical conditions. This survey was an outcome of understanding DS and many of its associated conditions and to emphasis the role of GC for parents and DS patients. From this survey about 34 percent of the parents have either a neutral or a disagreement over GC which must change. In developing nations particularly, efforts must be taken to create awareness regarding GC. The outlook and understanding in parents must be evolved in order to benefit the community and the nation. The clinical condition of every DS child is varied and parents must be aware of the conditions to be able to diagnose, manage appropriately and without delay. They have to understand that DS individuals can live a close to normal life with proper health care and better education and training. To raise a child with special-needs, parents have to adapt to varying requirements. Educating parents is of supreme importance to help in managing DS and, for them to be aware of the different outcomes. Emotional and behavioral problems are coped better with behavioral therapy and counseling.

It is impossible to achieve complete recovery, but the management is symptomatic. With appropriate and effective medical support most of the DS individuals can perform normal routine activities pertaining to their life without depending on other individuals. The perception and knowledge of the DS screening is important not mainly for clinicians' level but also for general public to improve diagnosis and management. In the current Indian scenario, it is all the more important for the people to gain awareness about DS and to eliminate the stigma related to GC so as to consider it as a means of professional help that can be offered when required. The survey has helped us understand the parents' perspective and also provided us with an opportunity to communicate to them about the role of GC. We believe, surveys of this kind can take professional help to various parts of the country and offer better medical support to the family.

RECOMMENDATIONS

This questionnaire may be employed as a routine clinical operating procedure in cases of familiar DS in genetic clinics.

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CONFLICT OF INTEREST

There was no conflict of interest declared.

ABBREVIATIONS

DS	Down Syndrome
GC	Genetic Counselling
Ts21	Trisomy 21
DSCR	Down Syndrome Critical Regions
NIPT	Non-Invasive Prenatal Test
NT	Nuchal Translucency
ID	Intellectual Disability
ADHD	Attention Deficit Hyperactivity Disorder
RT	Robertsonian Translocation
CHD	Congenital Heart Diseases
VSD	Ventricular Septal Defect
cfDNA	Cell-free DNA
CVS	Chorionic Villus Sampling
FISH	Fluorescence In Situ Hybridization
MLPA	Multiple Ligation-dependent Probe Amplification
QF-PCR	Quantitative Fluorescent PCR

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