International Journal on Emerging Technologies **11**(2): 997-1004(2020)

ISSN No. (Print): 0975-8364
ISSN No. (Online): 2249-3255

Chromosomal Analysis and Parental Age Effect on Incidence of Down's Syndrome in the Population of Himachal Pradesh: A Pilot Study

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(Received 20 January 2020, Revised 14 March 2020, Accepted 16 March 2020)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Down's syndrome (DS) is the most commonly diagnosed congenital anomaly in oocytes, abortuses and human live borns which is caused by imbalance in gene dosage resulting from trisomy of human chromosome-21. This investigation had included 50 DS patients who were selected on the basis of cytogenetic confirmation and 50 apparently normal children as a control. Cultivation of peripheral lymphocytes was done by standard method of 72 hours. The accepted level of statistical significance was P<0.05. Severity was tested according to Stanford-Binet test which was conducted for every patient and alienated all cases into four categories i.e. mild (50-55 to 70), moderate (50-55), severe (20-25 to 35-40) and profound (<20-25). To delineate the effect of advanced parental age on Down's syndrome, logistic regression analysis was performed. Chromosomal analysis revealed that trisomy-21 is the most common cause of DS and found to be associated with mild to moderate degree of intellectual disability or MR. In univariate logistic regression analysis, both advanced maternal (odds ratios 1.168 or approx. 1.17; 95% confidence interval: 1.08-1.26; P-value <0.001) and paternal ages (odds ratios 1.186 or approx. 1.17; 95% confidence interval: 1.09-1.28; P-value <0.001) were found to be noteworthy predictors of DS. In a multivariate logistic regression analysis, significant interaction between maternal and paternal age was observed (odds ratio 0.978; 95% confidence interval: 0.96-0.99; P-value 0.005) which suggested that effects of maternal age and paternal age on increasing odds of DS were also dependent on each other in addition to their unique independent predictive effects and might correspond to a paradigm for other genetic anomalies in children of fathers with advanced age. This investigation is a preliminary study to unravel the concealed facts about causes and risk factors of Down's syndrome individuals of Himachal Pradesh. Most of the studies of DS have considered maternal age as a risk factor but the studies to find the paternal age effect are lesser. This investigation has concluded that both maternal and paternal age act as risk factors for origin of this abnormality not only individually but in combination which is a unique finding. Taken together, this study will help in providing more true information to families an prenatal diagnosis, proper prognosis, recurrence risks and promising management options for this abnormality.

Keywords: Trisomy-21, Translocation, Mosaicism, Maternal age, Paternal age, Regression analysis.

I. INTRODUCTION

Down's syndrome (DS) is the most frequently identified genetic reason for intellectual disability and is characterized by specific phenotypic disposition including developmental delay, mental impairment, unique facial features like epicanthic folds, small mouth, permanently open mouth, brachycephalic heads, upwards slanting palpebral fissures, loose skin at the back of the neck, flat nasal bridge, single crease in palm and small ears with hearing loss among set of congenital malformations in the human population [1-2]. The incidence of Down's syndrome ranges from 1 in 600 to 1 in1000 in live born infants [3-4] but in India, it's incidence is 1 in 1250 [3]. The presence of a supernumerary chromosome 21 (Trisomy- 21) is the reason behind the typical features of DS. Approximately, 95% of all live born DS have an extra copy of Ch 21 due to meiotic non-disjunction of the chromosomes during parental gametogenesis whereas 3-4% Down's syndrome cases with relevant unbalanced translocation of Ch 21 and another to Ch14 and 1-2% persons with somatic mosaicism with two cell lines i.e. cell line with trisomy-21 and normal cell line, due to mitotic errors during embryonic development [5, 6]. Parental age is identified as potential risk factor for Down's syndrome in many investigations. It is a well established fact that the frequency of Down's syndrome rises dramatically with maternal age but much remains to be learnt about the paternal age effect on this chromosomal disorder. A few studies suggest a correlation of paternal age on this abnormality effect [7, 8] but conventional wisdom has held that increased risk for advanced paternal age is due to high correlation between fathers and mothers age and simply a reflection of the maternal age effect [9, 10]. In 95% of cases with Down's syndrome, meiotic non-disjunction of maternal origin is the cause of having extra 21 chromosome and the non-disjunction occured during the first meiotic division during oogenesis [6, 11]. The recognized pregnancies of trisomy-21 increases from 2% for maternal age below 25 years to 10% for mother of 36 years and to 33% by the age of 42 years [12]. Non-disjunction of paternal origin accounts for 5-10% of all trisomic cases [13, 14]. The degree of mental impairment ranges from mild to moderate, severe cases are rarely present [15]. The present investigation was aimed to examine the incidence of Down's syndrome causes and its association with maternal and paternal age in the population of Himachal Pradesh. In this study, cytogenetic investigation results reported the cases of trisomy and translocation only not the mosaics cases which is in contrast to previous reports [22, 28-31]. We defined the parental age effect on Down's syndrome and clarified whether a paternal age effect exists as a risk factor or not because in previous studies, paternal age is

not considered individually as a risk factor for DS. The present investigation is the first report in this regard from Himachal Pradesh.

II. SUBJECTS AND METHODS

The present study has been conducted on 50 children, 36 males and 14 females (sex ratio, 2.6:1), aged 5-18 yrs suspected to have Down's syndrome
i. e. showed the clinical features consistent with Down's syndrome and were subjected to complete morphological and cytogenetic analysis. Degree of mental retardedness was tested according to Stanford-Binet test which was conducted for every patient and alienated all cases into four categories i.e. mild (50-55 to 70), moderate (50-55), severe (20-25 to 35-40) and profound (<20-25). To dismiss or confirm Down's syndrome diagnosis and determine the type of aneuploidy, cytogenetic analysis was performed on 50 Down's syndrome and 10 normal individuals. Blood samples were collected in sodium heparin vacutainer. Chromosomal preparations were made by using standard culture technique with modifications [16-18]. A proforma, which incorporated pedigrees, course of pregnancy, parental age at the birth of child and other useful information, was filled for each patient after consulting their parents. Slides were stained with Giemsa stain and well spreaded plates were selected for karyotyping. Images were taken by Leica Image analyzer and karyotypes were prepared manually. Karyotypes were prepared according to instruction and rules given by International System of Human Chromosomal Nomenclature (ISCN) [19]. The informed consent was signed by parents and ethical approval was taken for all performed procedures. Logistic regression analysis was performed to delineate the effect of advanced parental age and other parameters on this abnormality. Statistical investigation was done via SPSS software.

III. RESULTS

In present investigation, maximum numbers (42.2%) of Down's syndrome individuals were in the age group of 9- 14 years (Table 1). There are 36 males and 14 females with sex ratio 2.67: 1 among 50 karyotyped cases. Cytogenetic analysis revealed that the most common type of abnormality was free trisomy-21 in Down's syndrome individuals which was observed in 47 (94%) patients (Fig.1, 2), whereas translocation (46, XX, +21, t (21; 21) (q10; q10) in 3 (6%) patients. Stanford's Binet's test has confirmed mild to moderate degree of intellectual disability or MR in Down's syndrome individuals. Fifty percent individuals had mild mental retardation, 48% have moderate and 2% with severe mental retardation (Table 1).
Majority of the cases were first 31(62%) and second 15 (30%) in order in sibship. Maximum numbers of individuals 37 (74%) belong to socioeconomically less developed families. Following logistic regression output shows that odds of having Down's syndrome increased by nearly 17% for every one year increase in maternal age and this change was statistically significant (OR 1.168 or approx. 1.17;95% CI: 1.08-1.26; P-value <0.001).

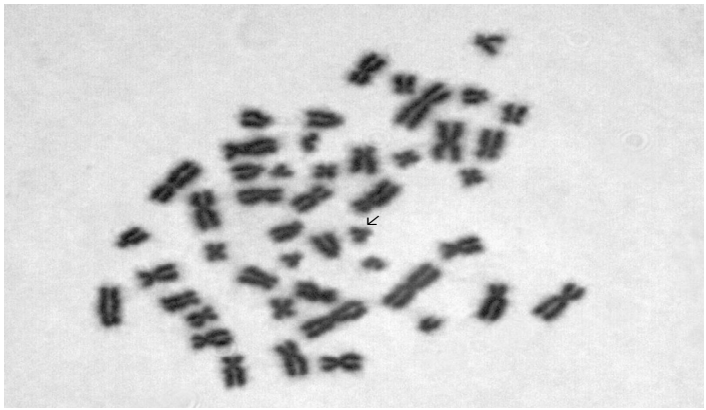

Fig. 1. Chromosomal plate used for preparation of karyotype.

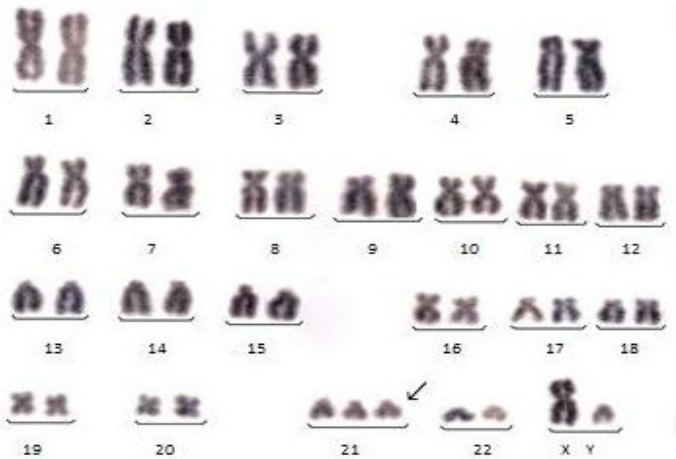

Fig. 2. Karyotype of the patient with 47, XY,+21 chromosomal constitution.

Table 1: Degree of mental retardedness in different age groups of Down's syndrome individuals.

Degree of MR	5-8 years	9-14 years	Above 14 years	%age
Mild	9	8	8	50
Moderate	9	12	3	48
Severe	-	1	-	2
Profound	-	-	-	-
%age	36	42	22	

Following logistic regression output shows that odds of having Down's syndrome decreased by nearly half (54% lower odds) for girl child in reference to male child and this change was statistically significant (OR 0.46;95% CI: 0.23-0.92; P-value 0.028). In addition, odds of developing Down's syndrome with increasing maternal age remained statistically significant when adjusted for confounding effect of gender(OR 1.155 or approx. 1.16;95% CI: 1.07-1.25; P-value <0.001).

Following logistic regression output shows that odds of having Down's syndrome increased by nearly 19% for every one year increase in paternal age and this change was statistically significant (OR 1.186 or approx. 1.19;95% CI: 1.09-1.28; P-value <0.001). In addition, odds of developing Down's syndrome with increasing paternal age remained statistically significant when adjusted for confounding effect of gender(OR 1.17;95% CI: 1.08-1.27; P-value <0.001). In a multivariate logistic regression analysis, odds of developing Down's syndrome were nearly significantly higher with both increasing paternal age (OR 2.12; 95% CI: 1.34-3.35; P-value 0.001) as well as maternal age (OR 1.95;95% CI: 1.24-3.06; P-value 0.004) when predictors of maternal age, paternal age and gender were simultaneously analyzed. Interestingly, there was significant interaction between maternal and paternal age as well (OR 0.978; 95% CI 0.96-0.99; P-value 0.005) suggesting that effects of maternal age and paternal age on increasing odds of Down's syndrome were also dependent on each other in addition to their unique independent predictive effects. However, lower odds of developing Down's syndrome with female gender lost its independent statistically significance when adjusted for maternal and paternal age (OR 0.55; 95% CI: 0.26-1.18; P-value 0.127). Following output gives odds of developing Down's syndrome with every one year increase in maternal age only for boys. The odds of having Down's syndrome in boys increased by nearly 14% for every one year increase in maternal age and this change was statistically significant (OR 1.137 or approx. 1.14; 95% CI: 1.04-1.23; P-value 0.003). Mean maternal age was found to be 28.38 ± 4.5 years.

Mean maternal age in males was found to be 28.13 ± 3.6 years. Mean maternal age in females was found to be 28.43 ±4.2 years. Following output gives odds of developing Down's syndrome with every one year increase in maternal age only for girls. The odds of having Down's syndrome in girls increased by nearly 23% for every one year increase in maternal age and this change was statistically significant (OR 1.1226 or approx. 1.13;95% CI: 1.03-1.46; P-value 0.02). The odds of having Down's syndrome in boys increased by nearly 12% for every one year increase in paternal age and this change was statistically significant (OR 1.119 or approx. 1.12;95% CI: 1.03-1.21; P-value 0.005). The odds of having Down's syndrome in girls increased by nearly 17% for every one year

increase in paternal age and this change was statistically significant (OR 1.771 ;95% CI: 1.26-2.47; P-value 0.001).

Probability Plots for developing Down's syndrome: Following probability plot (Fig.3) shows probability of developing Down's syndrome plotted on Y-axis (from 0 to 1 i.e. from 0% to 100%) and predictor of maternal age (in years) plotted on X axis. It is clearly evident that with increasing age, probability of developing Down's syndrome also showed nearly a linear increase, more noticeable after the age of 30 years. The probability plot of Down's syndrome against maternal age shows point estimates of probability (as blue squares) and their corresponding 95% Confidence Interval error bars as well. Following probability plot (Fig. 4) shows probability of developing Down's syndrome plotted on Y-axis (from 0 to 1 i.e. from 0% to 100%) and predictor of paternal age (in years) plotted on X axis. It is clearly evident that with increasing age, probability of developing Down's syndrome also showed nearly a linear increase, more noticeable after the age of 35 years. The probability plot of Down's syndrome against paternal age shows point estimates of probability (as blue squares) and their corresponding 95% Confidence Interval error bars as well.

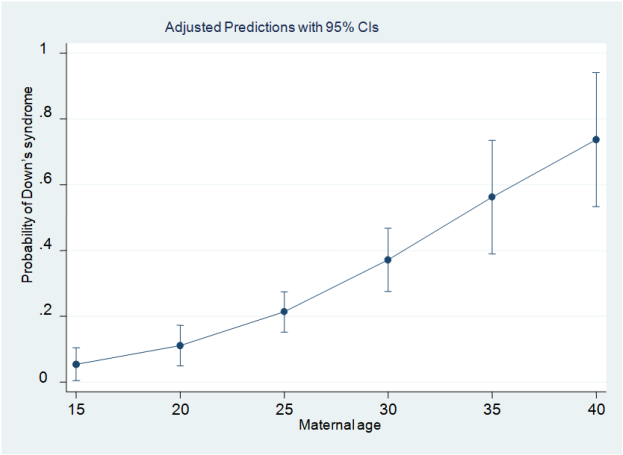

Fig. 3. Probability plot for developing Down's syndrone with increase in maternal age.

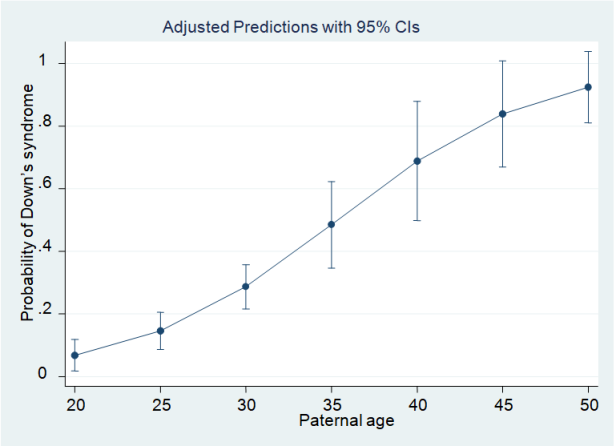

Fig. 4. Probability plot for developing Down's syndrone with increase in paternal age.

IV. DISCUSSION

In present investigation, 50 suspected to have Down's syndrome were analyzed cytogenetically from various districts of Himachal Pradesh. Free trisomy -21 is the most prevalent variant of Down's syndrome whose frequency varies between 83.82% to 95.52% [20-23].The reported incidence in present study is 94% which is in line the studies done before. Most frequent exchange in Robertsonian translocation occurs between non-homologous chromosomes which involves either two acrocentric chromosomes of D group (chromosomes 13-15) or G group (21 and 22), or between a D and a G group. Among them, 50% of these translocations have *de novo origin* and remaining 50% are inherited from a carrier parent (usually the mother). The most common translocation involved is between 14 and 21 which was followed by translocation between two 21 chromosomes [24]. Robertsonian translocation was observed in only in 1 case with incidence as 2%, which was in between two 21 chromosomes. The frequency of this variant falls near the reported range (2.66- 5.1%), small difference in percentages may occur due to the number of metaphases evaluated [25-27]. Our results are also in congruence with reports that identified above mentioned rearrangements as the most frequent translocations associated with trisomy 21. The parents of the case with translocation had a normal karyotype which indicates the *de novo* origin of the translocation. In this study, Robertsonian translocation is followed by mosaicism in terms of incidence i.e. 2.3% which also lied within the range reported from different parts of the world (1.19-10.78%) [22] [28-30] and have milder physical features as compared to trisomy-21 [31]. Inequalities in socio-economic status and mother's education are well established risk factors of Down's syndrome prevalence [32]. In this study 37 (74%) individuals were from socioeconomically deprived regions and most of the mothers have elementary education only. Low socioeconomic status and maternal education are also significantly correlated with other chromosomal abnormalities like deletions, ring chromosomes, duplication etc. in mentally retarded individuals [33-34]. To model advanced maternal and paternal age effects, there exists more than enough prior support as autonomous random walks for a wide variety of conditions and to identify the potent risk which are attributable to parental ages is necessary from epidemiologic perspective. Maternal age and paternal ages have high correlation with incidence of Down's syndrome due to strong independent effect of maternal age [35-37], it becomes very difficult to detect paternal age effect which is not secondary to an association with maternal age. When maternal age effect is controlled, the effects of paternal age changed to a small sparing risk and suggest the high correlation between maternal and paternal age which disguised the actual paternal age effect [38]. Risk of having Down's syndrome increases six times in couples older than 40 years as compared to those with less than 35 years [39].

In some epidemiological studies, paternal age association was observed [40] [7-8] while in several others, no significant effect was found on Down's syndrome [39, 41-45]. Disparities in these studies were mainly due to different sample sizes and use of different methods of statistical analysis [39]. The risk increases to six times in couples older than 40 years than in younger couples i.e. <35 years [40]. In current study, a small but significant sparing effect of paternal age was observed which is a novel finding and in line with many previous reports [7-8] [46]. Mean maternal age was found to be 32 ± 3.6 years. Most of the cases with free trisomy -21 had maternal age above 30 years but the cases with translocations and mosaicism had mothers of younger age [4]. This study had also hinted the *de novo* origin in the case with Robertsonian translocation because the parents had normal karyotype and were of younger age i.e. <30.

A report from Atlanta study on the incidence of DS had revealed that the prevalence of Down's syndrome was 8.5 per 10,000 for younger mothers and 55.3 per 10,000 for older women above 35 years age [47]. Similarly, a study on 52,965 amniocentesis had also suggested that the rate of trisomy- 21 increases with increase in maternal age above 35 years [48-49]. In this investigation, no effect of birth order was observed on Down's syndrome as most of the individual 31(62%) were first in their order in sibship [49]. The degree of mental impairment ranges from mild to moderate, severe cases are rarely present [19]. Chromosomal non-disjunction could be the reason behind Down's syndrome cases with advanced maternal age [50], this is determined by DNA polymorphic markers and analysis of chromosome heteromorphims error [51-53].

Table 2: Frequencies of different variants of trisomy -21 in previous reports.

Authors	Region	Total no. of cases	Free Trisomy- 21	Robertsonian Translocation	Mosaic trisomy-21
Mokhter et al. 2003	Egypt	673	642 (95.7%)	18 (2.7%)	5 (0.7%)
Devlin et al. 2004	Ireland	208	197 (94.7%)	3 (1.45%)	8 (3.8%)
Ahmed *et al.* 2004	Rawalpindi, Pakistan	295	282 (95.6%)	11 (3.7%)	2 (0.7%)
Azman *et al.* 2007	Malaysia	149	141 (94.6%)	1 (0.7%)	7 (4.7%)
Amayreh *et al.* 2009	Jordan	80	74 (92.5%)	2 (2.5%)	3 (3.8%)
Jayalakshamma *et al.* 2010	Karnatka, India	874	759 (86.9%)	77 (8.8%)	38 (4.3%)
Podder *et al.* 2012	West Bengal, India	85	78 (91.8%	2 (2.4%)	5 (5.9%)
Kolgechi *et al.* 2013	Kosova Albanian population	305	285 (93.4%)	17 (5.6%)	3 (1%)
Das et al. 2015	Dibrugarh, Assam	32	29 (90.63%)	1 (3.13%)	2 (6.25%)
Belmokhtar *et al.* 2016	Tlemcen, Algeria	22	20 (91%)	1 (4.5%)	1 (4.5%)
Pandey *et al.* 2018	Luckhnow, India	46	40 (93%)	2 (4.7%)	1 (2.3%)

Physiological time line of ovary and testis determine the basis of abnormalities which occurs due to meiotic errors in parents of advanced age [54]. The extended meiotic arrest i.e. in prophase of meiosis I and metaphase of meiosis II, leads to accretion of certain toxic effects which include environmental insults, hormonal imbalance and suboptimal ovarian functioning [51]. A report from Atlanta suggested that incidence specific to maternal age for live births with trisomy-21 is more in MII as compared to MI [55]. It has been suggested that despite of chronological age of women, biological aging of ovary is the main reason behind emergence of trisomy-21 in live births and two contrasting views support this. The first view found correlation between decrease in antral follicle count which accompany the reduction in total oocyte pool leads to hormonal imbalance in ovary and further aneuploid conception [56-58]. The second concept is limited oocyte pool hypothesis, which suggested that antral follicles are lesser in no. among older women [59]. Due to degradation of components of ovarian proteins that are utilized for chromosomal disjunction is the most appropriate interpretation for biological aging. Availability of antral follicles is limited among older women and ovary has to compromise in selecting a erroneous and suboptimal oocyte for ovulation [51]. If the origin of extra chromosomes no. 21 is due non-disjunction which occurs during spermatogenesis [60-61] ,mitotic errors which occurs after zygote formation and a de novo origin i.e. translocation [62], adverse effect of higher age is not apparent. The cause behind biological aging is genetic aging of mothers not the chronological aging [63] that means mothers with advanced age who have Down's syndrome offspring are "*genetically older*" than those who have euploid offspring at the same age. This was ascertained by estimating the telomere length (TL) of mothers cases by stratifying them by their age of conception (young, <29 years; middle, 29-35 years; and old,>35 years) and the stage of non-disjunction. Telomeric loss was observed as the age of mother progresses and is more in mother group with meiosis II errors [64]. The system which maintains telomeres is linked with chromosomal separating system at molecular level. If this molecular link will be degraded, it may affect both the systems all together. In mouse models, BubR1 gene was found to be the potent candidate as mutation in this gene leads to aneuploidy and senescence [65]. Sherman et al. (1994) had hypothesized that the reduction in recombination frequency is strongly associated with conception of the trisomy 21 at advanced maternal age however the incidence of tetrads without chiasmata remain most common in young mother [66-67]. Apart from reduced recombination, chiasma which is sub-optimally placed (pericentromeric exchange) is another reason behind chromosome 21 non-disjunction in mothers with advanced age and also apparent in model organisms such as *Caenorhabditis elegans* [68], *Drosophila* [69-71] and yeast. This occurs due to down regulation of centromeric complex, shugoshin that helps in cohesion and other spindle proteins [72]. On the other hand, effect of the environmental factor in inducing telomere loss at advanced mother age might concurrently have an effect on the chromosome segregation system in oocyte [73-76]. Whereas, paternal derived non-disjunction cases divulged a reduction in recombination frequency in MI cases and increase in pericentromeric exchanges in MII cases [77-78], moreover there is no delay in male meiosis as all the events of male meiosis are completed in puberty.

V. CONCLUSIONS

This investigation was carried out to report the incidence of different variants of Down's syndrome by comparative cytogenetic evaluation using classical karyotyping techniques and also to study the parental age effect on Down's syndrome in different districts of Himachal Pradesh. This study concluded that free trisomy-21 is the most common chromosomal variant that is followed by translocation and cases with mosaicism were not reported. Trisomy -21 is most common both in mother and father of advanced age i.e. >35 years. Majority of the DS patients are first in their order in sib-ship. Low socioeconomic status and maternal education are also significantly correlated with this chromosomal abnormality. Most of the patients have mild mental retardation followed by moderate and severe. No profound cases were reported. Both maternal and paternal ages were found to be the reasons behind emergence of this disorder, individually as well as mutually. In translocation cases, both parents were young. This investigation is a preliminary study in Himachal Pradesh and will help in understanding the prenatal diagnosis, the basis of inheritance and risk analysis of Down's syndrome. Even though advanced

parental age has been identified unequivocally as a risk, but its molecular relation with chromosome segregation system is still indecipherable. Further studies are required to unravel the aetiology of parental chromosome 21 non-disjunction and subsequent birth of Down's syndrome individuals. Cytogenetic techniques will always remain indispensable tool for diagnosis of chromosomal disorders and these disorders subsist as nature's guide to the molecular basis of many unexplained human disorders indicating possible treatment and management.

ACKNOWLEDGEMENT

Author is thankful to all participants and their parents for their co-operation throughout this study. Author is thankful to Department of Human Genetics, G. N. D. University, Amritsar for providing lab facility. Author is also thankful to H.P. University to provide financial assistance in the form of H.P.U JRF/SRF during this tenure.

Conflict of Interest. Author has no any conflict of interest.

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How to cite this article: Thakur N. (2020). Chromosomal Analysis and Parental Age Effect on Incidence of Down's Syndrome in the Population of Himachal Pradesh: a Pilot Study. *International Journal on Emerging Technologies*, *11*(2): 997–1004.