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RESEACH ARTICLE

IMPACT OF ADVANCED MATERNAL AGE ON FREE TRISOMY 21: INSIGHTS FROM KANO STATE, NIGERIA

Miko, A.M.[1*], Musa, S.A[-1], Mustapha, M[1], Anyiam, J. O[2], Timbuak, J. A[3], Adamu, L.H[4], and Jahun, S.M[5]

[1]Department of Human Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria, Nigeria
[2]Department of Paediatrics, Faculty of Clinical Sciences, Ahmadu Bello University, Zaria,
[3]Department of Human Anatomy, Faculty of Basic Medical Sciences, Yusuf Maitama Sule University, Kano, Nigeria
[4]Department of Human Anatomy, Faculty of Basic Medical Sciences, Federal University Dutse, Nigeria
[5] Department of Pediatrics, Aminu Kano Teaching Hospital, Kano, Nigeria

Abstract

Background Down syndrome (DS) is a prevalent genetic disorder caused by chromosomal abnormalities, primarily free trisomy 21. While advanced maternal age is a well-documented risk factor for DS, data specific to Northwestern Nigeria is limited. This study aimed to investigate the association between maternal age and the various cytogenetic types of DS in Kano State, Nigeria. **Materials and Methods:** The study involved purposively selecting and karyotyping 16 DS cases identified through peripheral blood samples at the Centre for Genetic Studies, Maulana Abul Kalam Azad University, Kolkata, India. Maternal and paternal ages were recorded, and statistical analyses were performed using Pearson's Chi-square test, independent samples, and receiver operating characteristic (ROC) curves to evaluate the significance of age as a risk factor. **Results:** The mean maternal age of mothers with DS was 35.66 ± 8.53 years, significantly higher than that of non-trisomic controls (31.28 ± 5.96 years; p = 0.002). All cases were identified as free trisomy 21, with no translocation or mosaic DS instances. The study found that maternal age was a significant risk factor (AUC=0.67, p=0.025), with a cut-off of 42 years indicating high risk. Paternal age did not show a significant correlation with DS risk. **Conclusion:** The findings confirm that advanced maternal age is a significant risk factor for free trisomy 21 in Kano State, reflecting global trends. However, the absence of other cytogenetic types suggests a need for broader research across Nigeria. Enhanced prenatal screening and genetic counselling are recommended for older mothers to manage DS risk better and inform preventive strategies.

Keywords: Cytogenetic Analysis, Down Syndrome, Karyotyping, Maternal Age, Trisomy 21

INTRODUCTION

Down syndrome (DS) is one of the most common genetic disorders, characterized by intellectual disability and various physical abnormalities (Antonarakis *et al*., 2020). The genetic basis of DS is linked to three cytogenetic mechanisms: free trisomy 21, translocation trisomy 21, and mosaic trisomy 21 (Esbensen *et al*., 2024), with free trisomy 21 accounting for the majority of cases. Although the mechanisms driving the inheritance of DS are not fully understood, advanced maternal age has been consistently identified as a significant risk factor. Studies

* Corresponding Author: Dr. Miko, A. M.
Tel: +234 8035682913
E-mail: rzimit19@gmail.com.

show that the risk of giving birth to a child with DS increases with maternal age, from 1 in 1,400 in women under 25 to 1 in 350 at age 35 and to 1 in 12 by age 49 (Esbensen *et al*., 2024; Laignier *et al*., 2021; Antonarakis *et al*., 2020).

Interestingly, despite this well-established risk, some research has indicated that up to 80% of DS cases occur in younger mothers under the age of 30 (Bull *et al*., 2022; Antonarakis *et al*., 2020). Most cases of DS result from nondisjunction during meiosis, with 90-95% of errors occurring in maternal meiosis and 3-5% from paternal errors (Olagunju *et al*., 2021). Nondisjunction is most common in the first meiotic division (80%) compared to the second (20%) (Pal *et al*., 2021). While advanced maternal age is a known factor, the prevalence of DS in younger women suggests additional genetic or environmental influences (Bull *et al*., 2022).

Numerous studies conducted outside Nigeria have explored the association between maternal age and the cytogenetic types of DS (Esbensen *et al*., 2024; Laignier *et al*., 2021; Antonarakis *et al*., 2020). In developed countries, extensive research has consistently shown a strong link between advanced maternal age and an increased likelihood of free trisomy 21, the most common type of DS (Antonarakis *et al*., 2020). For example, a large-scale study in the United States revealed that women over the age of 35 were at a significantly higher risk of giving birth to a child with DS compared to younger women, with the risk increasing exponentially as maternal age advances (Antonarakis *et al*., 2020). Similarly, European and Asian studies have confirmed that advanced maternal age correlates with nondisjunction errors during meiosis, particularly in the first meiotic division, which results in trisomy 21 (Kaur and Kaur, 2020). However, while these studies provide a robust understanding of DS risk in older mothers, some reports have also noted that a large proportion of DS cases occur in younger women, suggesting that maternal age alone may not fully explain the variability in DS prevalence (Bull *et al*., 2022; Antonarakis *et al*., 2020).

While the link between advanced maternal age and DS is well-documented globally (Olagunju *et al*., 2021; Pal *et al*., 2021; Antonarakis *et al*., 2020), there is little or no data to the best knowledge on this association within the context of Northern Nigeria, particularly Kano State. The region's unique genetic and cultural factors necessitate targeted research to understand how maternal age influences this population's prevalence and cytogenetic types of DS. Without this understanding, there is a risk of underestimating the need for prenatal screening programs and failing to provide adequate care for high-risk pregnancies.

This study aimed to fill the knowledge gap by exploring the relationship between maternal age and DS in Kano State. The findings will provide critical insights into local risk factors, aiding healthcare professionals in implementing more effective prenatal screening and intervention programs. Additionally, understanding the role of maternal age in DS risk can help guide genetic counselling methods and inform public health strategies to reduce the incidence of DS in Northern Nigeria.

The main objective of this study was to examine the association between maternal age and the cytogenetic types of DS, remarkably free trisomy 21, in a population from Kano State, Nigeria. The study also assessed the role of paternal age and other potential risk factors, contributing to a more comprehensive understanding of DS etiology in the region.

Materials and Methods

Study Design and Setting

This study utilized a cross-sectional design and was conducted in Murtala Muhammad Specialist Hospital, Kano State, Nigeria, providing a regional sample representative of the Northwestern Nigerian population in collaboration with the Centre for Genetic Studies, Department of Biotechnology and Biological Sciences, Maulana Abul Kalam Azad University, Kolkata, India, where the karyotyping and cytogenetic analyses were performed and the study period spanned from 2017 to 2019.

Study Population

The study population comprised clinically suspected DS patients from Kano State, Northwestern Nigeria, along with their mothers and control subjects. Inclusion criteria required patients to exhibit phenotypic characteristics indicative of DS, and only mothers of DS patients with available maternal and paternal age data were included. Control subjects consisted of non-trisomic children and their parents, enabling a comparative analysis. Exclusion

criteria ruled out patients with incomplete or missing karyotype or age data and families with a history of genetic disorders other than DS.

Ethical Statement

Ethical approval was obtained from the Research Ethics Committee of Ahmadu Bello University, Zaria, Kaduna State (ABUCHUSR/2020/001), Hospital Management Board, Kano state (MMSHZ/0324/III/167) and institutional review boards Maulana Abul Kalam Azad University, Kolkata, India. Informed consent was secured from all participants' parents or legal guardians before sample collection and analysis. The anonymity and confidentiality of study data were guaranteed. The study complied with the Declaration of Helsinki for human research ethics.

Sampling Technique and Sample Size Determination

A purposive sampling technique was employed, selecting 25 patients clinically suspected of DS, from which 16 were randomly chosen for detailed karyotype analysis based on the availability of complete demographic and clinical data. Control mothers were selected from non-trisomic children of a similar demographic region for comparative analysis. The sample size was determined based on the prevalence of DS in the region and available patient data, ensuring sufficient power for statistical analysis.

Karyotyping/Cytogenetic Analysis

Karyotyping was performed using G-banded peripheral lymphocyte cultures following standard protocols (Campos-Galindo, 2020). Blood samples were collected from DS patients and processed to prepare slides for chromosomal analysis. The GTG banding method was used to identify chromosomal abnormalities, explicitly focusing on trisomy 21, translocation, and mosaic DS. Karyotyping results were analyzed to determine each patient's cytogenetic type of DS.

Data Analysis

The Shapiro-Wilk test was used to determine the normality of data. Descriptive statistics were used to summarize maternal and paternal ages for both DS and control groups. Comparative analyses, including independent sample t-tests and Pearson's Chi-square test, were conducted to assess the differences in parental ages between DS cases and controls. The Receiver Operating Characteristic (ROC) curve was used to evaluate the discriminative ability of maternal and paternal ages as risk factors for DS. Man-Whitney U test was used to compare parental age differences in the free trisomy 21 group, represented by the box-and-whisker plot. *P*-values of less than 0.05 were considered statistically significant. Data were analyzed using SPSS version 28.

Results

Figure 1 compares the average maternal ages of mothers who gave birth to DS children with those of mothers in the control group (mothers of non-trisomic children). The mean maternal age for DS cases is notably higher than that of the control group. Specifically, the mean age for DS mothers is 35.66 ± 8.53 years, whereas for the control group, it is significantly lower at 31.28 ± 5.96 years. This difference is statistically significant ($P = 0.002$), indicating that advanced maternal age is strongly associated with a higher likelihood of having a child with DS.

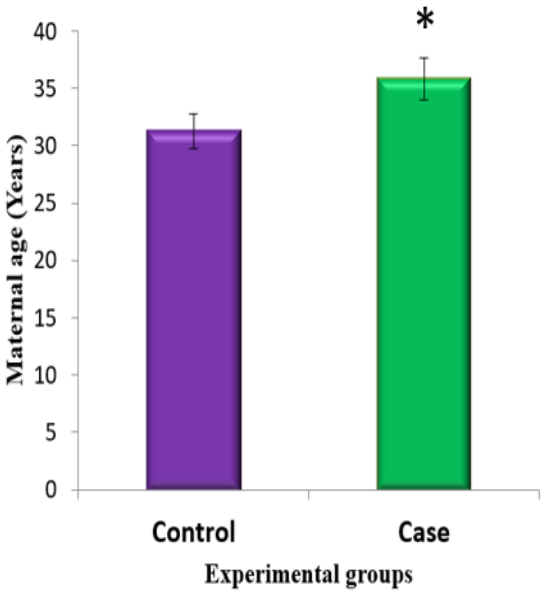

Figure 1: Comparison of mean maternal ages of control and DS cases. Values are expressed as mean ± SD. Control: Mothers of DS-free children; Cases: Mothers of DS children. * $P < 0.05$ compared with control.

Figure 2 also presents a comparison of maternal ages between mothers of children with DS and control mothers. The data is divided into five categories: mothers under 25 years (Group I), 25-30 years (Group II), 31-35 years (Group III), 36-40 years (Group IV), and those over 40 years (Group V). In Groups I and II, representing mothers younger than 30, there is a significant 80% increase in control mothers compared to DS mothers, suggesting a lower risk of DS in younger mothers. Conversely, in Groups IV and V (mothers older than 36), there is a marked increase in the number of DS mothers, with a 50% rise in the 36-40 age group and an 80% rise in those over 40. This pattern emphasizes the strong correlation between advanced maternal age—particularly beyond 35 years—and the risk of DS, confirming maternal age as a critical factor in DS occurrence.

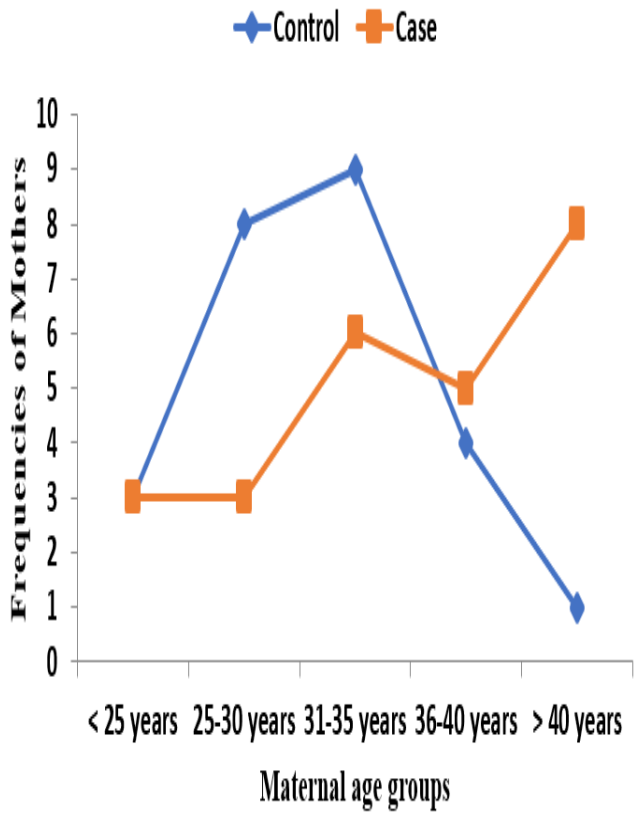

Figure 2: Comparison of the maternal age groups between control and case groups based on frequency. Chi-square value = 18.43, df = 4, P = 0.007. Control: Mothers of DS-free children; Cases: Mothers of DS children.

The ROC curve in Figure 3 illustrates the diagnostic ability of parental age (maternal and paternal ages) as a potential risk factor for distinguishing between having a child with DS and having a normal child (non-trisomic).

1. **Area Under the Curve (AUC) for Maternal Age**:

The AUC for maternal age is reported as 0.67, which indicates a moderate discriminatory ability. An AUC of 0.67 means maternal age has a 67% chance of correctly differentiating between a DS case and a non-DS case, suggesting that maternal age is a significant, though not perfect, predictor of DS risk.

2. **Cut-off for Maternal Age:**

A cut-off maternal age of 42 years and above is identified as a threshold for increased DS risk. With this cut-off, the sensitivity (the ability to correctly identify true positive DS cases) is 31%. In comparison, the specificity (the ability to correctly identify non-DS cases) is 100%. This indicates that while this age threshold has a low sensitivity (detects fewer DS cases), it is highly specific—meaning that mothers aged 42 and above have a very high likelihood of having a DS child compared to mothers younger than 42.

3. **AUC for Paternal Age:** The AUC for paternal age is reported as 0.62, indicating a weaker discriminatory ability than maternal age. Paternal age shows less of an impact as a risk factor for DS, with the AUC of 0.62 reflecting only a 62% chance of correctly distinguishing between DS and non-DS cases. The lower AUC value suggests paternal age is not a strong independent predictor of DS risk.

4. **Cut-off for Paternal Age:** The best cut-off for paternal age is reported as 47 years, with a sensitivity of 51% and a specificity of 84%. This indicates that while paternal age is not as strongly associated with DS as maternal age, older paternal age still shows a moderate association with DS risk, albeit with lower sensitivity than maternal age.

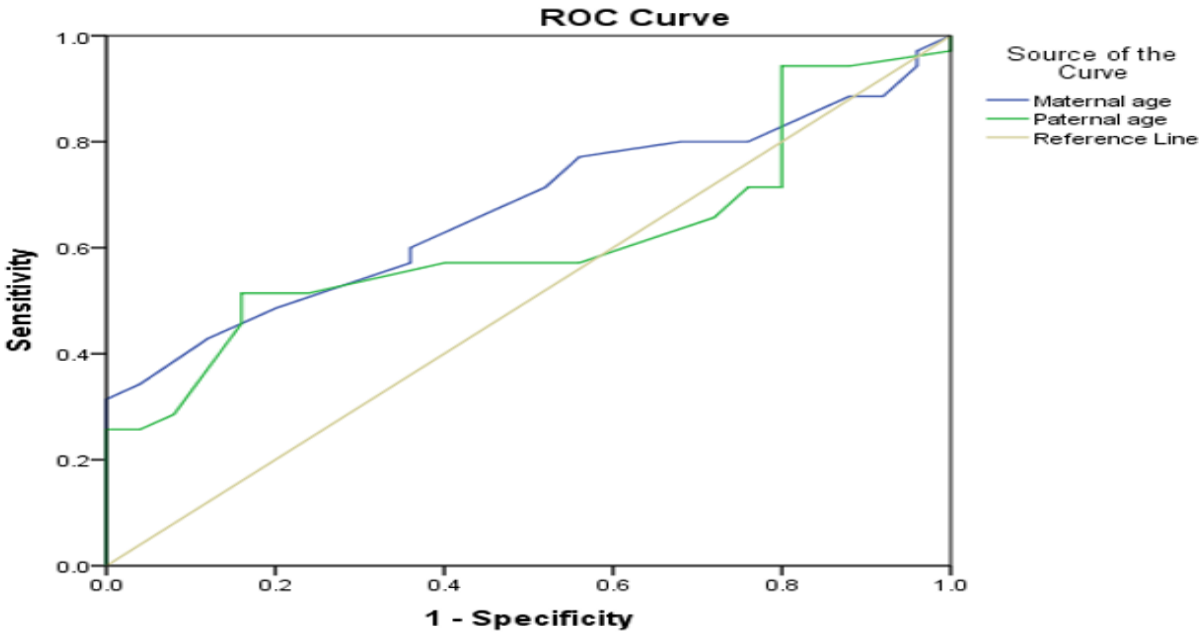

Figure 3: ROC Curve analysis of maternal and paternal age for predicting DS risk

Table 1 presents the karyotype results from the genetic analysis of 16 DS cases. The table focuses on identifying the specific chromosomal abnormalities associated with DS in these patients viz:

1. **Free Trisomy 21 (100%)**: All 16 cases (100%) showed Free Trisomy 21, meaning that an extra chromosome 21 was present in all the cells of these patients. This is the most common type of DS.

a. **47, XX, +21**: 7 out of the 16 cases (43.75%) were females, as indicated by the 'XX' sex chromosomes.

b. **47, XY, +21**: 9 out of the 16 cases (56.25%) were males, as indicated by the 'XY' sex chromosomes.

This shows that there is a relatively balanced gender distribution, with a slightly higher proportion of male DS cases.

2. **Translocation (0%)**: None of the 16 cases exhibited translocation trisomy. Translocation DS occurs when part of chromosome 21 attaches to another chromosome, but it was not observed in this sample.

3. **Mosaicism (0%):** Similarly, there were no cases of mosaic DS, where some cells have an extra chromosome 21 and others do not. Mosaic DS presents milder symptoms than full trisomy, but this was not detected in this sample.

Table 1: Distribution of karyotype results in DS Cases

Karyotype result	Number of cases	%
Free trisomy		
Total	16	
47, XX, +21	7	43.75
47, XY, +21	9	56.25
Translocation	0	0
Mosaic	0	0
Total	16	100

Figure 3 presents the comparison of parental ages (maternal and paternal) for cases of Free Trisomy 21:

1. **Maternal Age:** The median maternal age for cases of Free Trisomy 21 is 35.6 years with a standard deviation (SD) of ±8.53 years. The box-and-whisker plot shows the interquartile range (IQR) and the spread of ages, with the central bar representing the median. The figure indicates that most mothers of children with DS are in their mid-30s, with some variability extending

into older age groups. The p-value for maternal age is highly significant ($p < 0.0001$), indicating a strong association between advanced maternal age and the likelihood of giving birth to a child with Free Trisomy 21. This confirms that older maternal age is a significant risk factor for DS.

2. **Paternal Age:** The median paternal age for cases of Free Trisomy 21 is 46.34 years with an SD of ±11.7 years. The box-and-whisker plot shows that paternal age tends to be higher than maternal age, with a broader IQR, suggesting a wider age range for fathers. However, despite the spread, paternal age shows less statistical significance in this analysis compared to maternal age. Although paternal age is displayed, its p-value is less critical than maternal age (since it is less directly associated with DS risk). However, it is still presented for comparative purposes.

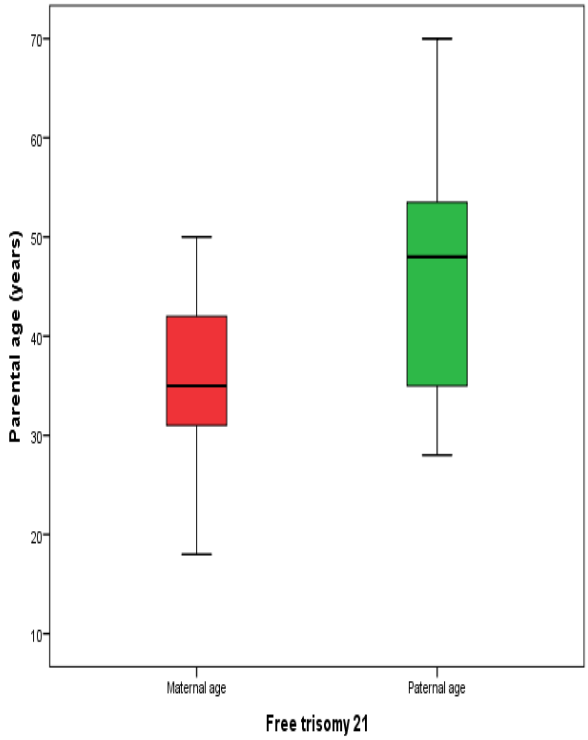

Figure 4: Box-and-Whisker plots of parental age distribution in free Trisomy 21: Median, Quartiles, and Statistical significance (Maternal Age = 35.6 ± 8.53, Paternal Age = 46.34 ± 11.7, $p < 0.0001$).

Discussion

This study found that maternal age is a significant risk factor for DS. The mean maternal age for mothers of DS children was 35.66 ± 8.53 years, notably higher than that of non-trisomic mothers (31.28 ± 5.96 years). This difference was statistically significant, reinforcing the established link between advanced maternal age and the likelihood of having a child with DS. In particular, 50% of the DS cases were from mothers aged 35 years and older, a finding consistent with studies from developed countries that have identified similar age-related risks for DS (Esbensen *et al*., 2024; Laignier *et al*., 2021; Antonarakis *et al*., 2020). Globally, the risk of DS has been shown to increase substantially after age 35 due to chromosomal nondisjunction, which is more frequent as maternal age advances (Aprigio *et al*., 2023; Bull *et al*., 2022).

Interestingly, while the study confirmed the well-known association between advanced maternal age and DS, it also revealed that a substantial proportion of DS cases occur in younger mothers. This is consistent with earlier reports from other regions, such as the United States, where some studies have found that up to 80% of DS cases occur in mothers younger than 30 (Chen *et al*., 2022; Antonarakis *et al*., 2020). The exact reasons for this observation are not fully understood. However, it may point to other genetic or environmental factors contributing to DS beyond maternal age (Antonarakis *et al*., 2020). The study results suggest that while age is a crucial factor, it is not the sole determinant of DS risk, indicating the need for further investigation into other potential risk factors.

The karyotype analysis showed that all 16 DS cases in the study were due to free trisomy 21, with no translocation or mosaic DS cases. This aligns with findings from international research, where free trisomy 21 accounts for most DS cases, typically over 90% (Antonarakis *et al*., 2020). However, the absence of translocation and mosaic cases in this study is notable, as other studies have reported lower, but still present, instances of these types (Chen *et al*., 2022). This discrepancy could be due to the sample size or regional genetic variations. It is possible that genetic counselling and screening practices in the population studied may not detect or report rarer forms of DS or that specific environmental or genetic factors in the region contribute to the higher prevalence of free trisomy 21.

The study also examined paternal age, with the mean paternal age for DS cases found to be 46.34 ± 11.7 years.

However, paternal age did not emerge as a significant risk factor for DS, contrasting with maternal age, which was strongly correlated with DS risk. Although some studies have suggested that advanced paternal age may contribute to the occurrence of DS (Kaltsas *et al*., 2023; Antonarakis *et al*., 2020), the findings here indicate that its role may be minimal. The lack of significance for paternal age as a risk factor could be due to a smaller contribution to nondisjunction events during meiosis or other genetic mechanisms (Antonarakis *et al*., 2020). This suggests that maternal age remains the primary concern in predicting DS risk, particularly in populations with a higher prevalence of older mothers during childbirth.

Clinically, these findings have important implications for prenatal care in Nigeria. The confirmation of maternal age as a significant risk factor for DS emphasizes the need for enhanced prenatal screening, especially for women over 35. Identifying a maternal age cut-off of 42 years as exceptionally high risk underscores the importance of early and accurate prenatal diagnostic tools such as karyotyping or non-invasive prenatal testing (NIPT). By prioritizing screening for high-risk mothers, healthcare providers can offer timely genetic counselling, thus improving pregnancy outcomes and reducing the incidence of DS. Furthermore, the absence of translocation and mosaic cases suggests that screening efforts may be focused on detecting free trisomy 21, simplifying risk management strategies in this population.

This study has several limitations, including its relatively small sample size of 16 DS cases, which may limit the generalizability of the findings to broader populations. Additionally, the focus solely on free trisomy 21, with no cases of translocation or mosaic types, may not fully represent the spectrum of DS. The study's regional scope—limited to Kano State—might not account for genetic and environmental variability across other areas of Nigeria. To enhance the robustness and applicability of future research, it is recommended to include larger and more diverse sample populations, incorporate a broader range of DS types, and expand the study to different regions of Nigeria. Additionally, integrating longitudinal studies could provide deeper insights into how maternal age interacts with other genetic and environmental factors in the development of DS.

Conclusion

This study confirms that advanced maternal age is a significant risk factor for DS in Kano State, Northwestern Nigeria, with a mean maternal age of 35.66 years observed in DS cases, significantly higher than that of non-trisomic controls. The results underscore the predominant role of free trisomy 21 as the primary cytogenetic type in this population, aligning with global findings and highlighting the need for further research into other genetic and environmental contributors. Given the identified risk, there is a pressing need for improved prenatal screening and genetic counselling for older mothers to manage better and mitigate the risks associated with DS. Expanding research to include larger and more diverse samples across different regions of Nigeria will provide a more comprehensive understanding of DS risk factors and help develop targeted prevention strategies.

Conflict of Interest

The authors declare that they have no conflicts of interest

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Authors' contributions:

MAM: Drafted the original manuscript, MSA; JOA and TJA: proofread the manuscript MM and ALH: Data analysis and JSM: Assisted with karyotyping.

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