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Original Article

Screening for Down Syndrome in Pregnant Women in Anar County Using Serum Markers: Alpha-Fetoprotein, Pregnancy -Associated Plasma Protein A, and hCG

Mohaddeseh Soleymani [1], Kamran Vosoo [2*]

[1] Shahid Bahonar University of Kerman Master of Science in Cell and Molecular Biology Specialization: Genetics
[2] Department of science, Faculty of biology, Payame Noor University, Qeshm, Iran

*** Corresponding Author:** Kamran Vosoo, Department of science, Faculty of biology, Payame Noor University, Qeshm, Iran. E-mail: kamranvosoo1@gmail.com

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Abstract

Introduction: Down syndrome (DS), caused by trisomy 21, is a common chromosomal abnormality associated with intellectual disability, developmental delays, and characteristic physical features. Prenatal screening enables early risk assessment and informed decision-making. Advanced maternal age is a major risk factor, highlighting the need to consider maternal age alongside biochemical and ultrasound markers. This study investigated the association between maternal age and key prenatal markers—including Pregnancy-Associated Plasma Protein A (PAPP-A), β-human Chorionic Gonadotropin (β-hCG), Alpha-Fetoprotein (AFP), Inhibin A, unconjugated Estriol (uE3), and Nuchal Translucency (NT)—and evaluated their contribution to Down syndrome risk stratification across maternal age groups.

Methods: A cross-sectional study was conducted on 138 pregnant women referred to a diagnostic laboratory in Anar County, Kerman Province. Serum markers were measured in first- and second-trimester screenings, alongside ultrasound assessment of NT. Statistical analyses, including one-way ANOVA, examined associations between maternal age, markers, NT, and estimated Down syndrome risk.

Results: Maternal age was significantly associated with estimated Down syndrome risk, highest among women aged 40–45 years and lowest in those aged 20–25 years. AFP and uE3 demonstrated the strongest predictive associations with risk, whereas PAPP-A, β-hCG, and NT contributed less prominently.

Conclusion: Combined prenatal screening using maternal serum markers and NT improves early identification of pregnancies at increased risk for Down syndrome. Integrating maternal age with biochemical screening optimizes risk stratification, especially in regional programs. Further multicenter studies are recommended to confirm and expand these findings.

Keywords: Down Syndrome, Prenatal Screening, Maternal Age, PAPP-A, β-hCG, Alpha-Fetoprotein

Introduction

Down syndrome (DS), caused by trisomy 21, is the most common chromosomal abnormality compatible with life and is associated with intellectual disability, developmental delays, and characteristic phenotypic features.[1] Globally, the prevalence of DS ranges from approximately 1 in 700 to 1 in 1,000 live births, with some regional studies reporting rates as high as 1 in 700.[2,3] The incidence of trisomy 21 increases with maternal age, from 1 in 800 at age 30 to 1 in 32 at age 45,[4] making maternal age one of the most important risk factors for fetal aneuploidy.[5]

Early prenatal screening for DS is crucial for timely risk assessment and informed clinical decision-making.[6] Screening is typically performed during the first and second trimesters using a combination of maternal serum markers and ultrasound parameters, with additional options including non-invasive prenatal testing (NIPT).[7] Maternal serum markers commonly evaluated include Pregnancy-Associated Plasma Protein A (PAPP-A), β-human Chorionic Gonadotropin (β-hCG), Alpha-Fetoprotein (AFP), Inhibin A, and unconjugated Estriol (uE3), while ultrasound focuses on nuchal translucency (NT).[8,9] These markers are selected based on their established associations with trisomy 21 and their ability to improve risk stratification across maternal age groups.[10,11]

Screening recommendations now include all pregnant women, regardless of maternal age, with abnormal serum marker results (e.g., risk of 1 in 375 or higher) prompting confirmatory diagnostic procedures such as amniocentesis.[12] Integrating maternal age with biochemical and ultrasound markers enhances the predictive accuracy of prenatal screening programs.[13,14]

Historically, Down syndrome was first described by John Langdon Down in 1866, based on observed phenotypic characteristics.[15] In 1956, Tjio and Levan confirmed the chromosomal basis of trisomy 21.[16] The most significant clinical manifestations remain intellectual disability and characteristic physical anomalies.[17] Overall, combining maternal age, serum markers, and NT measurement provides an effective framework for identifying pregnancies at increased risk of Down syndrome, supporting early interventions and counseling.[18]

Materials and Methods

Study Design and Participants

This cross-sectional study was conducted in Anar County, Kerman Province, Iran. A total of 138 pregnant women referred to Dr. Gholamreza Anarki Mohammadi's laboratory were enrolled between 2022-2024. Inclusion criteria included singleton pregnancies with confirmed gestational age based on the last menstrual period and ultrasound. Exclusion criteria were multiple pregnancies, known genetic disorders in the family, chronic maternal illnesses, or incomplete laboratory.

Prenatal Screening and Sample Collection

Maternal serum markers were measured during the first trimester (weeks 10–13) and second trimester (weeks 15–20) of pregnancy. First-trimester screening included free hCGβ, PAPP-A, and NT ultrasound measurements, while high-risk pregnancies underwent second-trimester quadruple tests, including AFP, uE3, Inhibin A, and total hCGβ.

Venous blood samples were collected using standard phlebotomy procedures, centrifuged, and serum aliquots were stored at 2–8 °C for up to 2 days or −20 °C for longer periods. During NT ultrasound, participants were instructed to have full bladders for optimal imaging, and measurements of nuchal fold thickness and crown-rump length were performed by experienced sonographers.

Measurement of Serum Markers

All assays were conducted using commercially available diagnostic kits according to the manufacturers' protocols: hCGβ and total hCG: Demeditec (Germany) and Diasorin (Italy) kits, AFP: Kavagi (Sweden), uE3 and Inhibin A: Demeditec and Asleib (Germany). Each assay followed standard ELISA procedures, including addition of standards, controls, and samples to wells, incubation, washing, addition of enzyme conjugates and substrates, and optical density measurement at 450 nm. For PAPP-A, hCGβ, AFP, and uE3, incubation times and washing steps were adjusted according to the manufacturer's instructions.

Data Collection

Maternal demographic and clinical data, including age, gravidity, parity, and gestational age, were recorded. All serum marker levels and NT measurements were used to estimate Down syndrome risk, stratified by maternal age groups.

Statistical Analysis

All analyses were performed using SPSS version 16 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± standard deviation and categorical variables as percentages. Associations between maternal age and individual serum markers, as well as NT, were evaluated using one-way ANOVA. Statistical significance was set at $P < 0.05$.

Results

Frequencies of Pregnant Women in Different Age Groups

In Chart 1, the frequency of each age group is displayed as a bar chart. A total of 138 pregnant women visiting a genetic laboratory in Anar between October 2019 and March 2020 were included in this study. The women were classified into six age groups: 15-20, 20-25, 25-30, 30-35, 35-40, and 40-45.

The number and percentage of pregnant women in different age groups participating in this study are shown in Table 1. The mean age of the pregnant women included in this study was 28.23 years (standard deviation = 5.79), with the youngest participant being 15.6 years old and the oldest being 42.6 years old.

According to investigations, the incidence of trisomy 21 increases with advancing maternal age, such that its

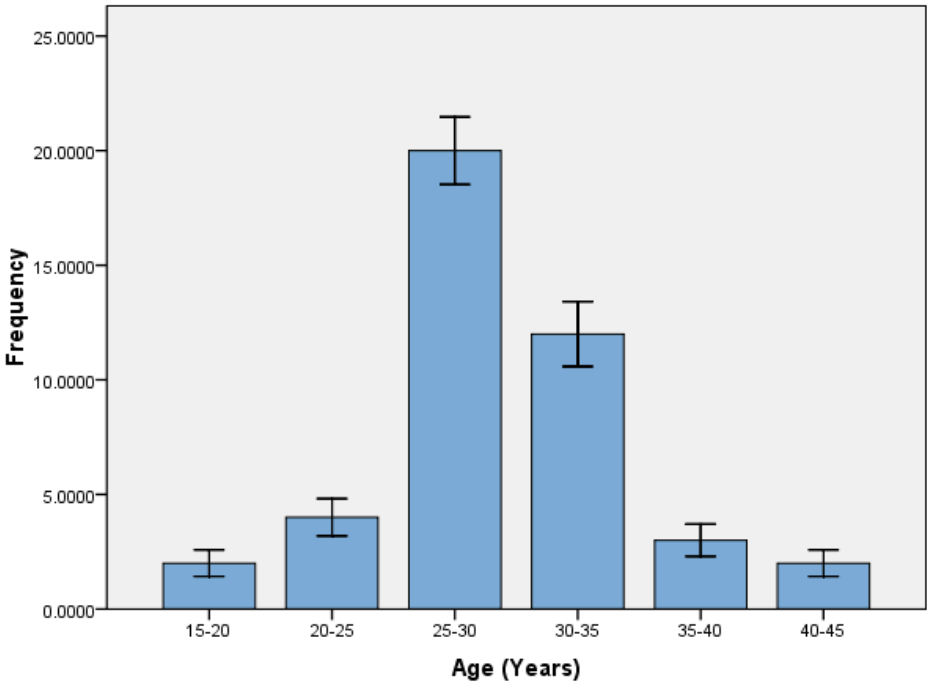

Chart 1. Frequency of Pregnant Mothers Included in the Study by Age Group.

Table 1. Statistical Analysis of the Mean Age of Pregnant Women in SPSS Software

Minimum	Maximum	Mean	Standard Deviation	Variance
15.600	42.600	28.233	5.79	33.525

prevalence at age 30 is 1 in 800, at age 35 is 1 in 386, at age 36 is 1 in 307, at age 37 is 1 in 242, at age 38 is 1 in 189, at age 39 is 1 in 146, at age 40 is 1 in 112, and at age 45 is 1 in 32.

In Table 2, the frequency, mean, and standard deviation of each age group of pregnant women studied are summarized. based on the present data, the highest number (46) of pregnant women who visited the laboratory, with a frequency percentage of (33.33), was in the age group of 25-30 years, while the lowest number (3) of pregnant women who visited the laboratory, with a frequency percentage of (2.17), was in the age group of 40-45 years.

Additionally, the screening test results for Down syndrome were positive in 13 out of 138 pregnant women who visited Dr. Gholamreza Anaraki Mohammadi's laboratory. Of these, 53.84% (7 individuals) were under 35 years old and 46.15% (6 individuals) were over 35 years old.

The NT values of the studied pregnant women in different age groups are as follows: The mean NT values obtained from the ultrasound of pregnant women included in this study was 1.15 with a standard deviation of 0.30, as shown in Table 3.

Table 2. Frequency of Participants Studied by Specific Age Groups

Standard Deviation	Mean	Percentage of Frequency	Frequency	Age Group
1/57	15/92	6/52	9	15-20
1/64	22/24	22/46	31	20-25
1/27	27/6	33/33	46	25-30
1/45	32/07	24/63	34	30-35
1/45	37/1	10/86	15	35-40
0/46	42/06	2/17	3	40-45

Table 3. Mean NT Values in Ultrasound of Pregnant Women Studied

	Sum of squares	Degree of freedom	Mean square	F test	Level of significance
Between Groups	693.454	5	138.691	0.637	0.672
Within Groups	14583.187	67	217.660		
Total	15276.641	72			

In the present study, no significant relationship was found between the thickness of nuchal translucency (NT) and the age groups of pregnant mothers (P = 0.67, F = 0.63). The mean NT in different age groups is presented in Table 4-4. Additionally, the mean NT for pregnant women younger and older than 35 years was (1.16 ± 0.30) and (1.12 ± 0.18), respectively, which did not show a significant difference (P = 0.63, F = 0.22).

The mean NT in pregnant women with healthy newborns and those at risk for Down syndrome was (1.14 ± 0.24) and (1.31 ± 0.48), respectively, indicating no significant difference in the nuchal translucency thickness between these two groups.

The mean nuchal translucency (NT) thickness in pregnant women of different age groups is shown in Chart 2.

The mean PAPP-A values in different age groups of the studied pregnant women were equal to 0.92 with a standard deviation of 0.11, as shown in Table 5.

Table 4. Mean Nuchal Translucency Thickness in Pregnant Mothers by Age Group

Standard Deviation	NT Mean (mm)	Age group
		15-20
0.06	1.24	20-25
		25-30
0.37	1.14	30-35
0.14	0.61	35-40
		40-45

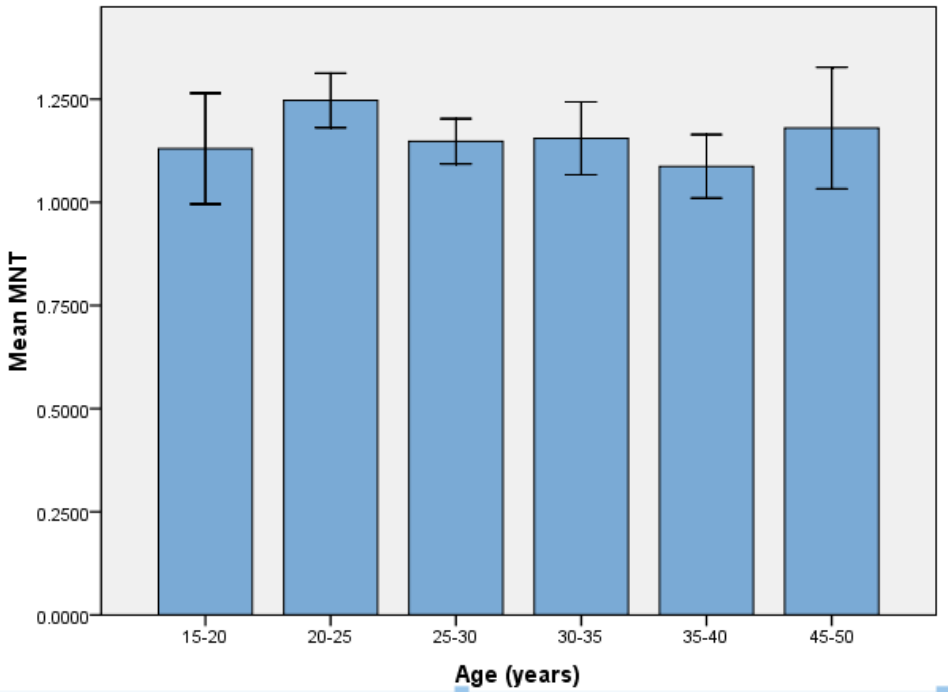

Chart 2. Mean Nuchal Translucency Thickness in Fetuses of Pregnant Mothers by Age Group.

In this study, no significant relationship was observed between the level of the PAPP-A hormone and the age groups of pregnant mothers (P = 0.537, F = 0.825).

The mean serum level of PAPP-A in different age groups is presented in Table 6. Additionally, the mean PAPP-A between pregnant women under and over 35

years was (1.12 ± 0.82 mg/l) and (0.77 ± 0.32 mg/l), respectively, which did not show a significant difference ($P = 0.27$, F = 1.23).

Furthermore, the mean PAPP-A in healthy pregnant women and those with newborns at risk for Down syndrome was (1.08 ± 0.80 mg/ml) and (0.84 ± 0.24 mg/ml), respectively, indicating no significant difference in the PAPP-A hormone levels between these two groups ($P = 0.403$, F = 0.707).

The mean values of PAPP-A in the serum of pregnant women expressed in micrograms per milliliter for the specified age groups are shown in Chart 3.

Table 5. Mean PAPP-A Values in Serum of Pregnant Women

Minimum	Maximum	Mean	Standard Deviation	Variance
0.563	1.312	0.110	0.270	0.073

Table 6. Mean Levels of PAPP-A in Different Age Groups of Pregnant Women

Standard Deviation	Mean PAPP-A (µg/ml)	Age Group
0/15	0/56	15-20
0/45	1/05	20—25
0/56	1/06	25-30
1/15	1/31	30-35
0/39	0/81	35-40
0/29	0/73	40-45

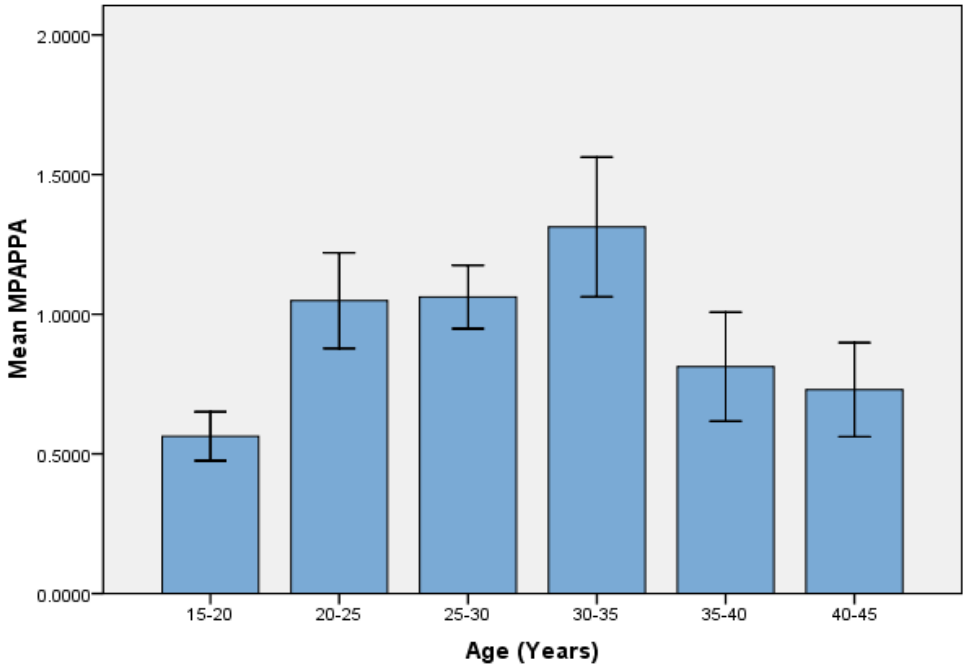

Chart 3. Mean Serum PAPP-A Levels in Pregnant Women by Age Groups.

Table S1. Mean Levels of β-hCG in the Serum of the Studied Pregnant Women

Minimum	Maximum	Mean	Standard Deviation	Variance
0.260	184.000	3.806	20.417	416.857

The levels of free βHCG in the serum of pregnant women studied in different age groups.

Table S1 shows that the mean serum level of free βHCG in the studied pregnant women was 3.81 (Standard Deviation = 2.28).

In the present study, no significant correlation was found between the level of free βHCG and the age of mothers. However, the highest level was observed in

the age group of 30-35 years, with a measurement of 31.1 Nano grams per milliliter. In contrast, the lowest mean (0.56) belonged to the age group of 15-20 years. The mean values of free βHCG in different age groups are presented in Table 8.

Additionally, the mean free βHCG levels for pregnant women under and over 35 years of age were 1.13 ± 0.82 Nano grams per milliliter and 0.78 ± 0.33 Nano grams per milliliter, respectively, showing no significant difference ($P = 0.673$, F = 18.0).

Table S2. Mean Serum Levels of Free β-HCG in Pregnant Women Across Different Age Groups

Standard deviation	Mean free βHCG (ng/ml)	Age group
0/15	0/56	15-20
0/45	1/05	20-25
0/56	1/04	25-30
1/15	1/31	30-35
0/39	0/81	35-40
0/29	0/73	40-45

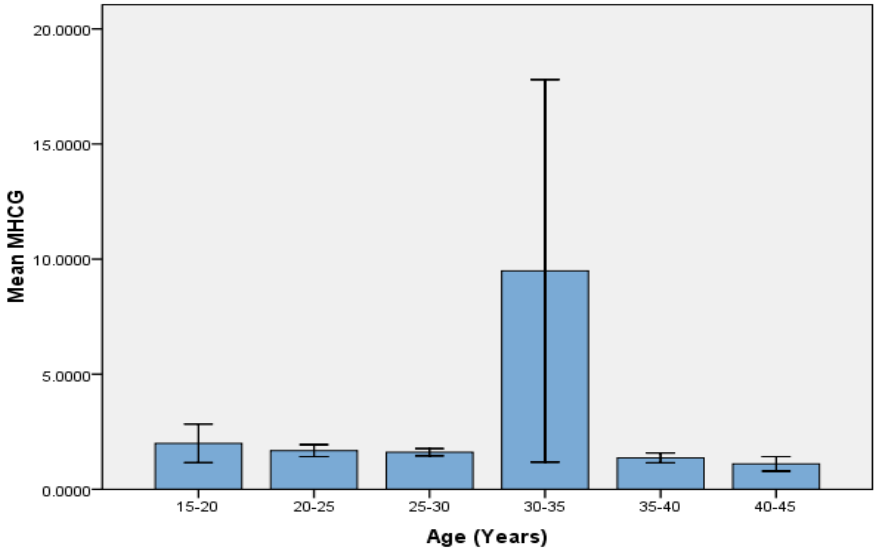

Chart 4. Mean Serum Levels of Free β-HCG in Pregnant Women Across Different Age Groups.

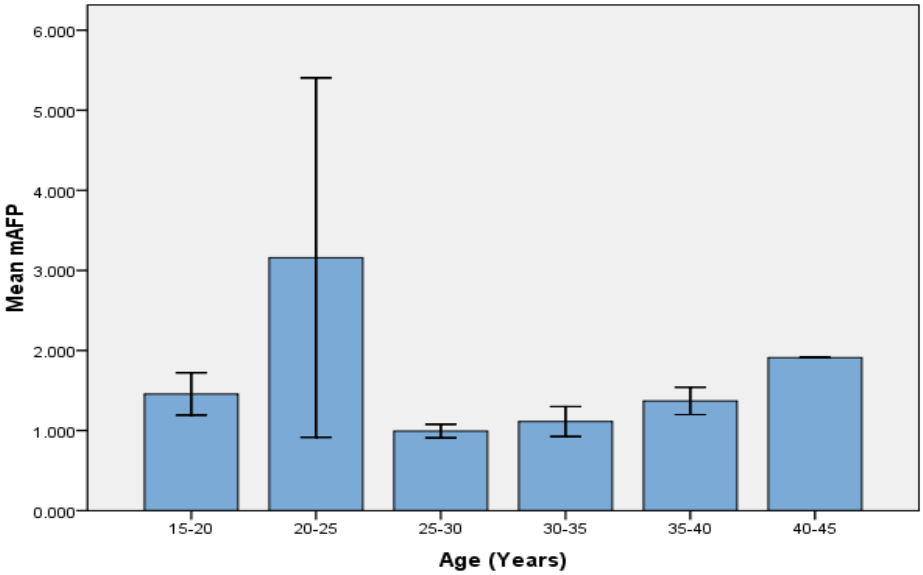

Chart 5. Mean Serum Levels of AFP in Pregnant Women Across Different Age Groups.

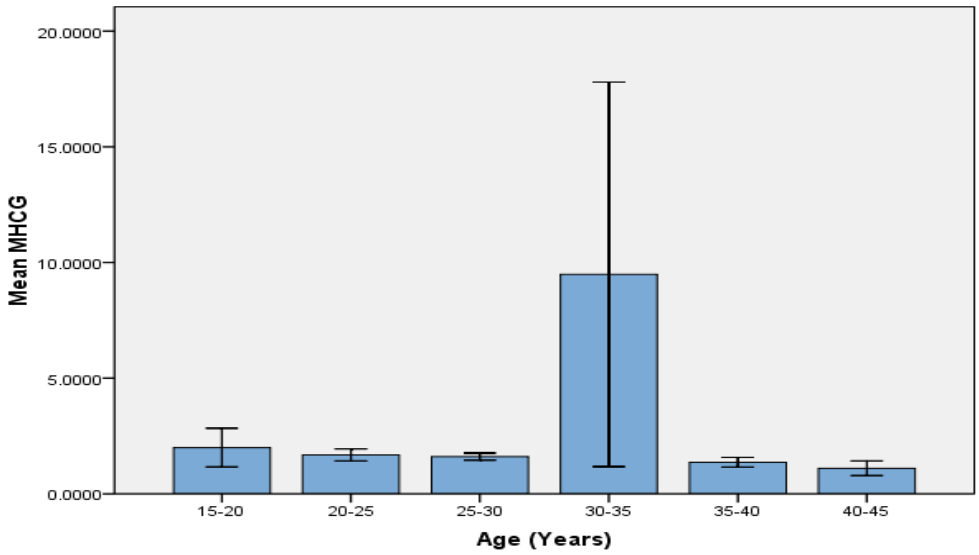

Chart 6. Mean β-HCG Levels in the Serum of Pregnant Women Across Different Age Groups.

Similarly, it can be seen from the table S2 the mean free βHCG levels in pregnant women with healthy infants and those with a risk of Down syndrome were 0.80 ± 1.09 Nano grams per milliliter and 0.85 ± 0.25 Nano grams per milliliter, respectively, indicating no significant difference between these two groups (P = 0.76, F = 0.94).

The mean levels of free βHCG in the serum of pregnant women in the specified age groups are illustrated in chart 4.

The mean serum levels of AFP in pregnant women across the specified age groups are illustrated in chart 5.

Chart 6: The mean serum levels of βHCG in pregnant women, measured in international units, across the specified age groups.

Discussion

In this study, we observed a clear association between maternal age and estimated risk for Down syndrome, consistent with prior research indicating that the probability of fetal aneuploidy increases with advancing maternal age. Among the evaluated markers, AFP and unconjugated estriol (uE3) showed stronger associations with maternal age, supporting their predictive value in prenatal risk assessment. This aligns with previous studies reporting that elevated AFP and altered uE3 levels are significant indicators of trisomy 21, particularly when combined with maternal age.[19]

Nuchal translucency (NT) measurement remains an important sonographic marker. Our findings indicated that NT and crown-rump length increase with maternal age, which is consistent with previous large-scale studies.[20] However, discrepancies exist: while NT combined with maternal age has been reported to detect up to 83% of Down syndrome cases in large cohorts, our data showed less pronounced differences, suggesting that NT alone may not reliably predict all cases, highlighting the importance of integrating multiple markers in screening algorithms.

…consistent with evidence from meta-analyses showing that combined first-trimester screening using maternal age, PAPP-A, free β-hCG and nuchal translucency increases Down syndrome detection rates compared with single markers alone.[20] Such variability across studies may result from population-specific factors, sample size differences, or methodological variations. The relative contribution of individual markers such as PAPP-A + free β-hCG has been reported to outperform single markers in first-trimester screening.[21]

Clinically, these findings reinforce the utility of combining maternal age, serum markers, and NT measurements to optimize prenatal Down syndrome screening in Anar County. By identifying high-risk pregnancies early, healthcare providers can offer timely counseling, targeted follow-up testing, and appropriate interventions, potentially improving maternal and fetal outcomes.[22]

Several limitations of this study should be acknowledged. First, the sample size was relatively small and drawn from a single laboratory, which may

limit the generalizability of the results to other regions. Second, not all high-risk pregnancies underwent confirmatory diagnostic procedures, such as amniocentesis, which may affect the precision of risk estimation. Finally, population-specific characteristics may influence marker performance, necessitating further multicenter studies to validate these findings.[23]

In conclusion, integrating maternal age with biochemical and ultrasound markers remains the most effective approach for prenatal screening of Down syndrome. Our results provide region-specific data that can guide screening strategies in Anar County and highlight the importance of systematic risk assessment to support early clinical decision-making.

Conclusion

Down syndrome is a genetic condition that cannot be cured, but prenatal screening and early care can assist families and affected individuals. Despite some controversies, screening methods including biochemical tests and ultrasound remain the best practices. Various trisomies account for approximately 25% of miscarriages and 4% of stillbirths. While there is no way to completely prevent Down syndrome, mothers can promote healthier pregnancies by taking multivitamins and avoiding smoking and alcohol. Previously, screening was only recommended for high-risk women, but age restrictions have become less stringent. Currently, there is no definitive non-invasive method for diagnosing Down syndrome in fetuses; thus, combined approaches continue to be the best available option. Increasing public awareness and paying attention to maternal mental health alongside screening is essential. Future studies should more closely consider the limitations of this research, including the status of the pregnant mother.

Conflict of Interest

The authors declare no conflicts of interest.

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