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Oxidative stress biomarkers as novel screening tools for trisomy 21: a case-control study

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Abstract

Objective Oxidative stress plays a pivotal role in the pathogenesis of Down syndrome (Trisomy 21), as chromosome 21 harbors multiple genes involved in redox homeostasis and antioxidant defense mechanisms. This study aimed to evaluate the roles of transcription factors nuclear factor erythroid 2-related factor 2 (NRF2) and nuclear factor-kappa B (NFKB), along with antioxidant enzymes cystathionine-γ-lyase (CSE) and NAD(P)H dehydrogenase [quinone] 1 (NQO1) in amniotic fluid (AF) and maternal serum (MS) as potential biomarkers for prenatal screening of Down syndrome (DS).

Methods This prospective case-control study included singleton pregnant women undergoing amniocentesis between 16 and 24 weeks of gestation at Haseki Training and Research Hospital, Istanbul. Participants were divided into two groups: 28 pregnancies with DS confirmed by karyotype analysis (DS group) and 37 pregnancies with normal karyotype results (non-DS group). Amniotic fluid and maternal blood samples were analyzed using enzyme-linked immunosorbent assay (ELISA) kits to measure the levels of selected biomarkers.

Results NQO1 levels were significantly higher in the DS group compared to the non-DS group in both amniotic fluid (924.84 ± 475.94 vs. 505.62 ± 358.17 ng/ml, $p < 0.001$) and maternal serum (716.216 ± 242.91 vs. 394.87 ± 344.86 ng/ml, $p < 0.001$). NRF2 levels were significantly lower in the DS group in both amniotic fluid (3.77 ± 4.20 vs. 6.47 ± 5.53 ng/ml, $p = 0.029$) and maternal serum (7.54 ± 5.68 vs. 14.46 ± 16.53 ng/ml, $p = 0.022$).

Conclusion The study highlights the importance of further research to validate the use of these antioxidant enzymes and transcription factors in non-invasive prenatal testing, which may reduce the need for invasive procedures and associated complications.

Clinical trial number Not applicable.

Keywords Prenatal diagnosis, Transcription factors, Antioxidant enzymes, Down syndrome, Amniocentesis, Oxidative stress markers

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Introduction

Amniotic fluid cells (AFC) are essential for prenatal screening to identify fetal abnormalities. During the second trimester, the concentration of these cells in the amniotic fluid might vary from 10 to 1000 cells per microliter. Amniotic fluid contains high levels of proteins that are influenced by genetics and the interaction between the fetus and the mother [32], and analyzing amniotic fluid could offer useful insights into the pathogenic processes crucial for embryonic development, especially since the pathology is not well characterized in humans [37].

Down syndrome (DS) is the most common human chromosomal abnormality caused by a trisomy of chromosome 21 [35]. It is characterized by congenital anomalies in fetuses, including congenital heart defects, gastrointestinal anomalies such as duodenal atresia, genitourinary system defects, and skeletal abnormalities s [8, 29], and poses a higher risk for postnatal mental retardation, intellectual disability, sleep apnea, thyroid disease, immune system defects, and ocular abnormalities [5, 16, 27]. Currently, the diagnosis of DS typically follows a two-step process: initial non-invasive screening (biochemical and genetic tests) to identify high-risk pregnancies, followed by confirmatory invasive diagnostic procedures (amniocentesis or chorionic villus sampling) in cases where screening indicates elevated risk. The diagnostic yield of invasive methods is 99.8% or higher, but these methods carry a 0.5-1% risk of miscarriage or fetal harm [11]. In contrast, noninvasive tests themselves are associated with 5–10% false positives [17] and therefore all positive results must be confirmed by invasive methods. To reduce or eliminate this false positive rate, new potential biomarkers of DS are needed that would enhance the accuracy of non-invasive testing. Extensive research is ongoing to identify more reliable biomarkers [7, 23, 39]. Some of these studies have also found that increased oxidative stress levels are associated with DS pathogenesis and therefore oxidative stress markers measured in DS individuals may be elevated. Considering that important genes of the oxidative stress pathway are located on chromosome 21 [2], it is warranted to evaluate oxidative stress biomarkers in prenatal screening.

Oxidative stress is characterized by an imbalance between oxidants and antioxidants, where the scales tip in favor of the oxidants. This imbalance can result in molecular damage and disrupt crucial oxidation-reduction (redox) signaling and regulatory mechanisms. Disruption of the redox signaling system leads to changes in the regulation of the redox potential within cells, resulting in redox stress caused by the production of reactive oxygen species (ROS). ROS and oxidative stress are harmful agents that can cause developmental abnormalities in the fetus by inducing structural changes during embryogenesis. Increased ROS production during organogenesis, when cells continue to differentiate, can cause structural anomalies [12].

The oxidative stress response system includes several key components: transcription factors that regulate gene expression and antioxidant enzymes that directly mitigate oxidative damage. Two critical transcription factors are nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor-kappa B (NF-κB). Important antioxidant enzymes include cystathionine-γ-lyase (CSE), which catalyzes the production of cysteine and plays a role in glutathione synthesis, and NAD(P)H dehydrogenase [quinone] 1 (NQO1), which converts quinones to hydroquinones and reduces ROS production [4, 34]. These components interact in complex regulatory networks to maintain redox homeostasis.

Utilizing innovative biochemical screening markers such as NRF2, NFKB, CSE, and NQO1 can enhance the accuracy and precision of noninvasive prenatal tests, leading to a reduction in unnecessary invasive procedures and minimizing the risk of miscarriage associated with such tests [1].

The following references guided our selection of oxidative stress markers for this study: (1) Barone et al. [2] demonstrated that chromosome 21 harbors genes involved in redox homeostasis, including SOD1, which affects oxidative stress levels in Down syndrome; (2) Perluigi et al. [25] reported altered Nrf2 pathway activity in Down syndrome; (3) Saha et al. [30] described the role of NF-κB in inflammation and its interaction with Nrf2; and (4) Zhang et al. [38] showed aberrant expression of oxidative stress markers, including NQO1, in pregnancy complications [2, 24, 30, 38].

Understanding the complex interplay between oxidative stress markers and transcription factors in Down syndrome could provide valuable insights for developing more accurate and less invasive diagnostic tools. However, the relationship between these biomarkers in amniotic fluid and maternal serum, and their potential diagnostic value in Down syndrome detection remains largely unexplored. Therefore, this study aimed to investigate the levels of antioxidant enzymes (CSE and NQO1) and their regulatory transcription factors (Nrf2 and NF-κB) in both amniotic fluid and maternal serum of pregnancies with and without Down syndrome.

Materials and methods

Study design

This prospective case-control study was carried out in the Perinatology Service of Istanbul Haseki Training and Research Hospital, affiliated with the University of Health Sciences, in the Sultangazi district of Istanbul, in 2024. After the purpose and nature of all the procedures employed were properly explained to each

pregnant woman, the participant was requested to sign an informed written consent form to participate in this study in accordance with the latest Declaration of Helsinki after the approval of Human Ethics Committee of our institution (Registration Number: 3-2024 dated January 23, 2024).

Participants

This prospective case-control study was conducted at the Perinatology Service of Istanbul Haseki Training and Research Hospital between January 2024 and June 2024. A total of 65 pregnant women who underwent routine amniocentesis between 16 and 24 weeks of gestation were included in the study. The study population was divided into two groups: 28 pregnancies with Down syndrome confirmed by karyotype analysis (DS group) and 37 pregnancies with normal karyotype results (non-DS group). The amniocentesis indications included advanced maternal age (≥ 35 years), abnormal first or second-trimester screening test results, abnormal ultrasonographic findings, and previous pregnancy or family history of chromosomal abnormalities.

Among the amniocentesis results, cases that revealed chromosomal abnormalities other than Down syndrome (including trisomy 18 [Edwards syndrome], trisomy 13 [Patau syndrome], Turner syndrome [45, X], and microdeletion syndromes) and multiple pregnancies, and mothers with systemic diseases (e.g., autoimmune disease, vasculitis, hemophilia, thrombophilia, HIV infection) were excluded from the study. Pregnant women with diabetes and hypertension were included in the study as these conditions are common in this population and excluding them would reduce generalizability of findings.

The sample size was calculated using G*Power software (version 3.1.9.7, Universität Düsseldorf, Germany). Based on preliminary data and relevant literature, with an α error of 0.05, power (1-β) of 0.80, and an effect size of 0.59, the minimum required sample size was calculated as 37 participants per group to detect significant differences between groups.

Clinical assessment

Detailed medical histories were obtained from all participants, including maternal age, gravidity, parity, previous pregnancy outcomes, and current pregnancy complications. All participants underwent detailed ultrasonographic examination prior to amniocentesis, including fetal biometry, anatomy scan, and assessment for markers associated with chromosomal abnormalities.

Diagnostic amniocentesis

Singleton pregnancies between 16 and 24 weeks of gestation with a high risk of chromosomal abnormalities and who accepted amniocentesis as a diagnostic test were included in our study. The complications that may occur during or after amniocentesis such as rupture of membranes, direct fetal injury, indirect fetal injury, infection, and fetal loss were explained to the patient and her husband and informed consent was obtained. Firstly, obsetric ultrasound was performed to determine fetal viability and position and placental location. Using a 20 gauge spinal needle, we performed the procedure under ultrasound guidance and continuous visualization of the needle throughout the procedure. For our study, we collected an additional 5–8 mL of amniotic fluid during amniocentesis and maternal blood samples afterwards. After centrifuging the amniotic fluid and maternal blood, we divided the serum samples into several equal portions into Eppendorf tubes and froze them at -80 °C until the Enzyme Linked Immunosorbent Assay (ELISA) was performed.

Biomarker analysis

Maternal blood and amniotic fluid samples were collected from each woman participating in this study. Maternal biochemical and hematological tests were performed. Serum Nrf2, NF-κB, NQO1 and CSE were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (BT LAB, China) according to the manufacturer's protocol. Serum samples were used undiluted. Standard solution was increased from 80 ng/ml starting concentrations to 2.5 ng/ml for Nrf2, from 12 ng/ml to 0.375 ng/ml for NF-κB, from 4000 ng/ml to 125 ng/ml for NQO1, from 80 ng/ml to 2.5 ng/ml for CSE, in the sample diluent supplied with the kit. The intra- and inter-assay coefficient of variation for assays in the Nrf2, NF-κB, NQO1 and CSE kits ranged between 8 and 10%.

Statistical analysis

Statistical analyses were performed using SPSS software version 28.0 (IBM Corp., Armonk, NY, USA). The normality of continuous variables was assessed using the Kolmogorov-Smirnov test. Continuous variables were presented as mean ± standard deviation, and categorical variables were expressed as numbers and percentages. Comparisons between groups were performed using Student's t-test for normally distributed variables and Mann-Whitney U test for non-normally distributed variables. Categorical variables were compared using Chi-square or Fisher's exact test, as appropriate.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of biomarkers. The area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and optimal cut-off values were calculated. Multiple logistic regression analysis was conducted to assess the independent associations between

Table 1 Maternal baseline clinical characteristics of two groups

Variables	Non-DS pregnancies (*n* = 37)	DS pregnancies (*n* = 28)	*P* value
Maternal age, years	32.54 ± 5.6	34.61 ± 7.49	0.227
Gravidity	3.24 ± 1.61	3.22 ± 1.37	0.955
Parity	1.81 ± 1.20	1.71 ± 1.24	0.754
Number of abortions	0.27 ± 0.56	0.64 ± 0.91	0.063
Number of living children	1.11 ± 0.99	1.04 ± 0.84	0.752
Model of delivery, n (%)			0.818
Nulliparous	5 (55.6%)	4 (44.4%)	
Vaginal Delivery	20 (60.6%)	13 (39.4%)	
Cesarean Section	12 (52.2%)	11 (47.8%)	
Diabetic pregnancy, n (%)			0.673
No	33 (57.9%)	24 (42.1%)	
Yes	4 (50.0%)	4 (50.0%)	
Hypertensive pregnancy, n (%)			0.398
No	36 (58.1%)	26 (41.9%)	
Yes	1 (33.3%)	2 (66.7%)	

Data were presented as mean with standard deviation and number (%) as appropriate

DS: Down syndrome

biomarkers and Down syndrome, adjusting for potential confounding factors. The model's performance was evaluated using the Hosmer-Lemeshow test for calibration and Nagelkerke R^2 for explanatory power.

A p-value < 0.05 was considered statistically significant. All statistical tests were two-sided.

Results

The study aimed to include 37 participants per group based on sample size calculations. While we successfully enrolled 37 pregnancies with normal karyotype results (non-DS group), we were only able to include 28 pregnancies in the DS group due to incomplete data collection for some eligible cases. Maternal baseline clinical characteristics of the participants are shown in Table 1. The mean maternal age was 34.61 ± 7.49 years in the DS group and 32.54 ± 5.6 years in the non-DS group ($p = 0.227$).

Obstetric characteristics including gravidity, parity, number of living children, and previous mode of delivery were similar between groups (all $p > 0.05$). The history of previous miscarriages tended to be higher in the DS group (0.64 ± 0.91 vs. 0.27 ± 0.56, $p = 0.063$). No significant differences were found in the rates of women with diabetes or hypertension between groups.

Ultrasonographic findings and fetal measurements of the study groups are presented in Table 2. The mean gestational age at amniocentesis was similar between groups (19.46 ± 2.56 vs. 19.08 ± 2.32 weeks, $p = 0.537$). No significant differences were observed in fetal biometric measurements including BPD, HC, AC, FL, and EFW between groups (all $p > 0.05$). The amniotic fluid index

Table 2 Ultrasonography findings of the participants

Variables	Non-DS pregnancies (*n* = 37)	DS pregnancies (*n* = 28)	*P* value
Gestational age at amniocentesis, weeks	19.08 ± 2.32	19.46 ± 2.56	0.537
BPD, weeks	18.89 ± 2.28	18.29 ± 2.79	0.545
HC, weeks	18.86 ± 2.36	18.96 ± 2.82	0.881
AC, weeks	18.73 ± 2.47	19.14 ± 2.99	0.555
FL, weeks	18.5 ± 2.6	18.7 ± 1.7	0.568
EFW, g	293.24 ± 157.937	272.82 ± 137.915	0.581
AFI, mm	44.16 ± 4.98	48.93 ± 10.44	0.032
Fetal cardiac anomalies*, n (%)			< 0.001
No	33 (75.0%)	11 (25.0%)	
Yes	4 (19.0%)	17 (81.0%)	
Extra-cardiac fetal anomalies‡, n (%)			< 0.001
No	34 (85.0%)	6 (15.0%)	
Yes	3 (12.0%)	22 (88.0%)	
Ultrasonographic soft markers§, n (%)			0.016
No	28 (68.3%)	13 (31.7%)	
Yes	9 (37.5%)	15 (62.5%)	

DS: Down syndrome, BPD: Biparietal diameter, HC: head circumference, AC: abdominal circumference, FL: femur length, EFW: estimated fetal weight, AFI: amniotic fluid index

*Cardiac anomalies included: Ventricular Septal Defect (VSD) with malalignment (most frequent), Atrioventricular Septal Defect (AVSD), Double Outlet Right Ventricle (DORV), and Tetralogy of Fallot (TOF)

‡Extra-cardiac Fetal Anomalies included: Ventriculomegaly (most frequent), duodenal atresia (characterized by "Double Bubble" Sign), esophageal atresia, encephalocele, omphalocele, cystic hygroma, dandy-walker malformation, hydronephrosis

§ Ultrasonographic Soft Markers: Nasal bone hypoplasia/absence, echogenic intracardiac focus, hyperechogenic bowel, Aberrant Right Subclavian Artery (ARSA), short femur length, short humerus length, Renal pyelectasis, Sandal gap deformity, Single umbilical artery, Clinodactyly

was significantly higher in the DS group (48.93 ± 10.44 vs. 44.16 ± 4.98 mm, $p = 0.032$).

Fetal cardiac anomalies were significantly more frequent in the DS group compared to the non-DS group (81.0% vs. 19.0%, $p < 0.001$), with VSD being the most common anomaly. Similarly, extra-cardiac anomalies were more prevalent in the DS group (88.0% vs. 12.0%, $p < 0.001$), with ventriculomegaly being the most frequent finding. Ultrasonographic soft markers were also more commonly observed in the DS group (62.5% vs. 37.5%, $p = 0.016$).

The amniotic and maternal serum biomarker levels of both groups are shown in Table 3. NQO1 levels were significantly higher in the DS group compared to the non-DS group in both amniotic fluid (924.84 ± 475.94 vs. 505.62 ± 358.17 ng/ml, $p < 0.001$) and maternal serum (716.216 ± 242.91 vs. 394.87 ± 344.86 ng/ml, $p < 0.001$).

NRF2 levels were significantly lower in the DS group in both amniotic fluid (3.77 ± 4.20 vs. 6.47 ± 5.53 ng/ml, $p = 0.029$) and maternal serum (7.54 ± 5.68 vs.

Table 3 Amniotic and maternal laboratory findings of participants

Variables	Non-DS pregnancies (*n* = 37)	DS pregnancies (*n* = 28)	*P* value
NQO1-AS (ng/ml)	505.62 ± 358.17	924.84 ± 475.94	< 0.001
NQO1-MS (ng/ml)	394.87 ± 344.86	716.216 ± 242.91	< 0.001
NFKB-AS (ng/ml)	2.51 ± 0.86	2.57 ± 0.75	0.783
NFKB-MS (ng/ml)	2.21 ± 1.45	2.80 ± 1.89	0.178
NRF2-AS (ng/ml)	6.47 ± 5.53	3.77 ± 4.20	0.029
NRF2-MS (ng/ml)	14.46 ± 16.53	7.54 ± 5.68	0.022
CSE-AS (ng/ml)	10.13 ± 7.89	12.21 ± 7.40	0.280
CSE-MS (ng/ml)	9.17 ± 11.02	16.68 ± 17.07	0.049

AS: amniotic serum, MS: maternal serum, NQO1: NAD(P)H dehydrogenase [quinone] 1, NRF2: nuclear factor erythroid 2-related factor 2, NFKB: nuclear factor-kappa B, CSE: cystathionine-γ-lyase, DS: Down syndrome

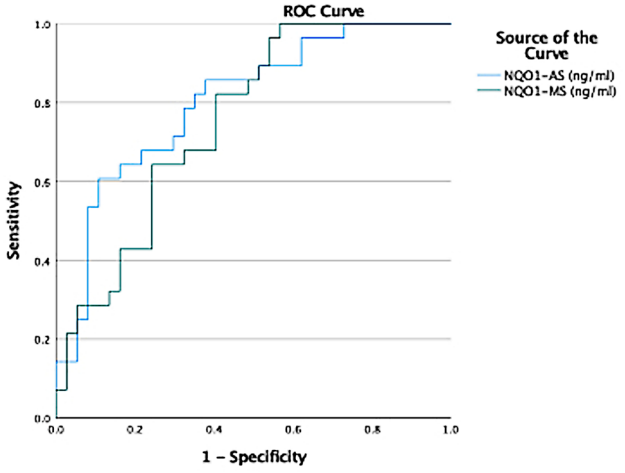

Fig. 1 Receiver Operating Characteristic (ROC) curves for NQO1-AS and NQO1-MS in predicting Down syndrome. The blue line represents NQO1-AS (AUC: 0.82, 95% CI: 0.74–0.90) and the green line represents NQO1-MS (AUC: 0.78, 95% CI: 0.69–0.87)

Table 4 Diagnostic performance of NQO1-AS and NQO1-MS for DS prediction

Parameter	NQO1-AS	NQO1-MS
AUC (95% CI)	0.82 (0.74–0.90)	0.78 (0.69–0.87)
Cut-off value (ng/ml)	800	650
Sensitivity (%)	85	80
Specificity (%)	75	70
Positive Predictive Value (%)	77	73
Negative Predictive Value (%)	83	78
Accuracy (%)	80	75

NQO1: NAD(P)H dehydrogenase [quinone] 1, AS: amniotic serum, MS: maternal serum, DS: Down syndrome, *AUC: Area Under the Curve*

14.46 ± 16.53 ng/ml, $p = 0.022$). CSE levels in maternal serum were significantly higher in the DS group (16.68 ± 17.07 vs. 9.17 ± 11.02 ng/ml, $p = 0.049$), while amniotic fluid levels showed no significant difference ($p = 0.280$). No significant differences were observed in NFKB levels between groups in either amniotic or maternal serum (all $p > 0.05$).

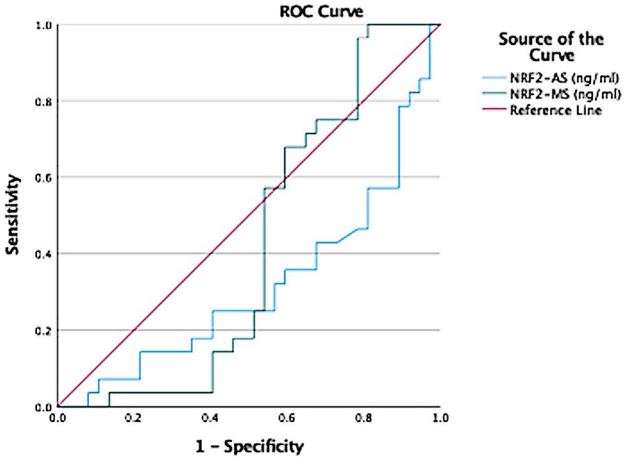

Fig. 2 Receiver Operating Characteristic (ROC) curves for NRF2-AS and NRF2-MS in predicting Down syndrome. The blue line represents NRF2-AS (AUC: 0.48, 95% CI: 0.39–0.57) and the green line represents NRF2-MS (AUC: 0.45, 95% CI: 0.36–0.54). The pink line indicates the reference line

Table 5 Diagnostic performance of NRF2-AS and NRF2-MS for DS prediction

Parameter	NRF2-AS	NRF2-MS
AUC (95% CI)	0.48 (0.39–0.57)	0.45 (0.36–0.54)
Cut-off value (ng/ml)	5.0	10.0
Sensitivity (%)	45	42
Specificity (%)	52	48
Positive Predictive Value (%)	48	45
Negative Predictive Value (%)	49	46
Accuracy (%)	47	44

AS: amniotic serum, MS: maternal serum, NRF2: nuclear factor erythroid 2-related factor 2, *AUC: Area Under the Curve*, *CI: Confidence Interval*, DS: Down syndrome

The ROC curve analysis and diagnostic performance parameters of NQO1-AS and NQO1-MS are presented in Fig. 1; Table 4. NQO1-AS demonstrated good diagnostic performance with an AUC of 0.82 (95% CI: 0.74–0.90). At the optimal cut-off value of 800 ng/ml, NQO1-AS showed 85% sensitivity and 75% specificity, with positive and negative predictive values of 77% and 83%, respectively. The overall accuracy was 80%.

Similarly, NQO1-MS showed favorable diagnostic capability with an AUC of 0.78 (95% CI: 0.69–0.87). Using a cut-off value of 650 ng/ml, NQO1-MS demonstrated 80% sensitivity and 70% specificity, with positive and negative predictive values of 73% and 78%, respectively. The overall accuracy for NQO1-MS was 75%.

The ROC curve analysis and diagnostic performance parameters of NRF2-AS and NRF2-MS are presented in Fig. 2; Table 5. NRF2-AS showed limited diagnostic performance with an AUC of 0.48 (95% CI: 0.39–0.57). At the cut-off value of 5.0 ng/ml, NRF2-AS demonstrated 45% sensitivity and 52% specificity, with positive and

negative predictive values of 48% and 49%, respectively. The overall accuracy was 47%.

Similarly, NRF2-MS showed limited diagnostic capability with an AUC of 0.45 (95% CI: 0.36–0.54). Using a cut-off value of 10.0 ng/ml, NRF2-MS showed 42% sensitivity and 48% specificity, with positive and negative predictive values of 45% and 46%, respectively. The overall accuracy for NRF2-MS was 44%.

The ROC curve analysis and diagnostic performance parameters of CSE-MS are presented in Fig. 3; Table 6. CSE-MS showed moderate diagnostic performance with an AUC of 0.62 (95% CI: 0.53–0.71). At the optimal cut-off value of 12.5 ng/ml, CSE-MS demonstrated 65% sensitivity and 60% specificity, with positive and negative predictive values of 62% and 63%, respectively. The overall accuracy was 62%.

Multiple logistic regression analysis results for predicting Down syndrome are presented in Table 7. The model demonstrated excellent overall fit (χ^2 test, $p < 0.001$) with a Nagelkerke R^2 value of 0.683, indicating that approximately 68.3% of the variance in Down syndrome prediction could be explained by the included biomarkers. The Hosmer-Lemeshow test ($p = 0.245$) confirmed good model calibration, and the model showed strong discriminative ability with an AUC of 0.842 (95% CI: 0.762–0.922) (Table 8).

Among the biomarkers, NQO1-AS showed the strongest positive association with Down syndrome (OR: 3.82, 95% CI: 1.94–7.51, $p < 0.001$), followed by NQO1-MS (OR: 2.95, 95% CI: 1.48–5.87, $p < 0.001$). CSE-MS also demonstrated a significant positive association (OR: 1.89, 95% CI: 1.01–3.54, $p = 0.049$). In contrast, both NRF2-AS and NRF2-MS showed significant negative associations with Down syndrome (OR: 0.45, 95% CI: 0.22–0.92, $p = 0.029$ and OR: 0.52, 95% CI: 0.30–0.91, $p = 0.022$, respectively). No significant associations were observed for NFKB-AS, NFKB-MS, or CSE-AS (all $p > 0.05$).

Discussion

In this study, we demonstrated that both amniotic fluid and maternal serum levels of oxidative stress markers show distinct patterns in pregnancies with Down syndrome compared to normal pregnancies. Notably, we found significantly elevated levels of NQO1 in both amniotic fluid and maternal serum, decreased levels of NRF2, and increased CSE levels in maternal serum of Down syndrome cases. To our knowledge, this is the first study to comprehensively evaluate these antioxidant markers (NRF2, NF-κB, NQO1, and CSE) simultaneously in both amniotic fluid and maternal serum in the context of Down syndrome.

While several studies have investigated various oxidative stress markers for prenatal screening of chromosomal abnormalities [3, 26], the potential role of these

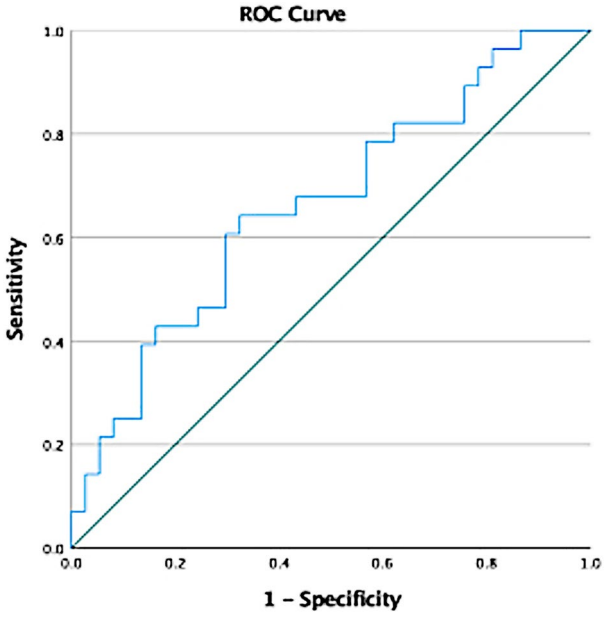

Fig. 3 Receiver Operating Characteristic (ROC) curve for CSE-MS in predicting Down syndrome. The blue line represents CSE-MS (AUC: 0.62, 95% CI: 0.53–0.71). The diagonal line indicates the reference line

Table 6 Diagnostic performance of CSE-MS for DS prediction

Parameter	CSE-MS
AUC (95% CI)	0.62 (0.53–0.71)
Cut-off value (ng/ml)	12.5
Sensitivity (%)	65
Specificity (%)	60
Positive Predictive Value (%)	62
Negative Predictive Value (%)	63
Accuracy (%)	62

CSE: cystathionine-γ-lyase, MS: maternal serum, *CI: Confidence Interval*, DS: Down syndrome, *AUC: Area Under the Curve*

Table 7 Multiple logistic regression analysis for DS prediction

Biomarker	Odds Ratio (95% CI)	*P* value	Standardized Coefficient
NQO1-AS	3.82 (1.94–7.51)	< 0.001	0.684
NQO1-MS	2.95 (1.48–5.87)	< 0.001	0.542
CSE-MS	1.89 (1.01–3.54)	0.049	0.328
NRF2-AS	0.45 (0.22–0.92)	0.029	-0.294
NRF2-MS	0.52 (0.30–0.91)	0.022	-0.276
NFKB-AS	1.12 (0.49–2.56)	0.783	0.048
NFKB-MS	1.45 (0.84–2.51)	0.178	0.156
CSE-AS	1.38 (0.77–2.48)	0.280	0.142

AS: amniotic serum, MS: maternal serum, NQO1: NAD(P)H dehydrogenase [quinone] 1, NRF2: nuclear factor erythroid 2-related factor 2, NFKB: nuclear factor-kappa B, CSE: cystathionine-γ-lyase, DS: Down syndrome, *CI: Confidence Interval*

Odds ratios are adjusted for all variables in the model

Table 8 Model fit and performance statistics of the multivariate logistic regression analysis

Performance metrics	Value	Interpretation
Overall Model Fit (χ^2 test)	$p < 0.001$	Significant model fit
Nagelkerke R^2	0.683	68.3% of variance explained
Hosmer-Lemeshow Test	$p = 0.245$	Good model calibration
AUC (95% CI)	0.842 (0.762–0.922)	Strong discriminative ability

Note AUC: Area Under the Curve; CI: Confidence Interval. The model demonstrates excellent overall fit ($p < 0.001$), with the Nagelkerke R^2 indicating that approximately 68.3% of the variance in Down syndrome prediction is explained by the included biomarkers. The non-significant Hosmer-Lemeshow test ($p > 0.05$) confirms good model calibration, and the AUC shows strong discriminative ability

specific antioxidant markers in Down syndrome detection has remained largely unexplored. Our findings not only provide new insights into the oxidative stress profile of Down syndrome pregnancies but also suggest potential novel biomarkers for non-invasive prenatal screening.

An intriguing finding of our study was the decreased levels of NRF2 despite elevated oxidative stress markers in Down syndrome pregnancies. Under normal physiological conditions, NRF2 acts as a master regulator of antioxidant response, becoming activated during oxidative stress to upregulate antioxidant gene expression [9]. However, our observation of reduced NRF2 levels, coupled with increased NQO1 and CSE levels, suggests a more complex regulatory mechanism in Down syndrome.

This paradoxical finding might be explained by the chronic nature of oxidative stress in Down syndrome [20]. Chronic oxidative stress, unlike acute stress conditions, may lead to adaptive mechanisms including the downregulation or exhaustion of the NRF2 pathway. Similar pathway exhaustion phenomena have been observed in other chronic conditions characterized by persistent oxidative stress [14, 19, 33]. The reduced NRF2 levels we observed might therefore represent an adaptive response to sustained oxidative stress rather than a primary defect.

In our study, we found increased levels of NQO1 in amniotic fluid and maternal serum and CSE in maternal serum. These antioxidant enzymes are known to be regulated through multiple pathways, including the NF-κB signaling cascade and various post-transcriptional mechanisms [15]. Although we did not observe significant changes in NF-κB levels in our study, other transcription factors or regulatory mechanisms may compensate for reduced NRF2 activity by maintaining or even increasing antioxidant enzyme expression as a protective mechanism against oxidative damage.

Our findings of decreased NRF2 levels seem to contradict previous studies suggesting an increased NRF2 response in Down syndrome. The BACH1/NRF2 axis has been extensively studied in Down syndrome and BACH1 encoded on chromosome 21 is known to negatively regulate NRF2 [25]. Pagnotta et al. [21] showed that BACH1 overexpression contributes to oxidative stress-induced damage by disrupting this balance. However, previous studies have identified that several genes involved in oxidative stress regulation are located on chromosome 21 [18].

Experimental studies have provided additional insights into the role of NRF2 in Down syndrome. Zamponi et al. [36], showed that NRF2 activation in Down syndrome mouse cells significantly reduced oxidative stress and restored mitochondrial function, highlighting its potential protective role. Our findings of decreased NRF2 levels along with increased NQO1 are in interesting contrast to studies in other conditions such as chronic kidney disease, where researchers found simultaneous decreases in both NQO1 and NRF2 levels [22]. These observations underscore the complexity of oxidative stress regulation in Down syndrome and suggest that the relationship between NRF2 and downstream targets may be context-dependent, particularly during fetal development.

The role of NF-κB in our study deserves particular attention, especially given its well-documented importance in embryonic development. NF-κB has been established as a crucial regulator of embryonic stem cell development, particularly in neural crest-derived and mesenchymal stem cells [6, 10]. Experimental studies in mice have provided valuable insights into NF-κB's role during embryonic development. For example, Torchinsky & Toder [31], demonstrated that NF-κB pathways influence embryonic sensitivity to developmental stresses, while Kim et al. [13], showed that NF-κB expression levels vary with developmental signals such as retinoic acid exposure.

Our findings of slightly elevated NF-κB levels in the amniotic fluid of Down syndrome cases, although not reaching statistical significance, align with these experimental studies. This trend might reflect the complex developmental abnormalities associated with Down syndrome and suggests that NF-κB upregulation could be a response to developmental stress. The observation that NF-κB levels can be higher during periods of embryologic developmental anomalies provides context for our findings, even though the changes we observed were modest.

These findings collectively suggest that the oxidative stress response in Down syndrome involves multiple pathways, with NRF2 showing decreased levels while NF-κB trends toward elevation. This pattern might represent a compensatory mechanism where different stress response pathways are differentially regulated to adapt to the chronic oxidative stress environment characteristic of Down syndrome.

Recent studies have provided important insights into the role of antioxidant enzymes in Down syndrome. A study in 2024 showed that children with Down syndrome exhibit elevated levels of several antioxidant markers, including CSE, due to an extra copy of the cystathionine beta synthase (CBS) gene on chromosome 21 [28]. Our findings are in agreement with these observations, showing increased CSE levels in both amniotic fluid (12.21 ± 7.40 vs. 10.13 ± 7.89 ng/ml, $p = 0.280$) and maternal serum (16.68 ± 17.07 vs. 9.17 ± 11.02 ng/ml, $p = 0.049$) of Down syndrome cases. Notably, while the increase in amniotic fluid CSE levels did not reach statistical significance, maternal serum CSE levels were significantly higher in Down syndrome cases compared to controls, with an approximately twofold increase.

In this study, we investigated the association between oxidative stress markers and Down syndrome by analyzing key antioxidant enzymes and transcription factors in both amniotic fluid and maternal serum. Our findings demonstrate significantly elevated NQO1 levels in both amniotic fluid and maternal serum of Down syndrome cases, coupled with decreased NRF2 levels and increased CSE levels in maternal serum. These results suggest a complex interplay between different oxidative stress pathways in Down syndrome pathogenesis.

Multiple logistic regression analysis revealed that NQO1, particularly in amniotic fluid, showed the strongest association with Down syndrome, demonstrating good diagnostic performance with high sensitivity and specificity. The concurrent finding of reduced NRF2 levels despite elevated NQO1 suggests the activation of alternative regulatory pathways in response to the chronic oxidative stress environment characteristic of Down syndrome.

Limitations

Our study has several limitations. The relatively small sample size (28 Down syndrome cases and 37 controls) may have limited our ability to detect smaller differences between groups and perform subgroup analyses. Being a single-center study may affect the generalizability of our findings to other populations. Additionally, the cross-sectional nature of our study prevents us from examining temporal changes in biomarker levels throughout pregnancy. Moreover, while we adjusted for some confounding factors in our analysis, we were unable to control for all potential maternal variables that might influence oxidative stress markers, such as dietary patterns, environmental exposures, and genetic variations in maternal antioxidant pathways. While we found significant associations between certain biomarkers and Down syndrome, further large-scale, multicenter studies are needed to validate our findings and explore their potential clinical applications in prenatal screening.

Conclusion

In conclusion, our study provides novel insights into the role of oxidative stress markers in Down syndrome and identifies NQO1 as a potential biomarker for prenatal screening. The strong diagnostic performance of NQO1, especially when measured in both amniotic fluid and maternal serum, suggests its potential value in enhancing current prenatal screening protocols. The unexpected finding of decreased NRF2 levels despite elevated oxidative stress markers warrants further investigation into the complex regulatory mechanisms underlying redox homeostasis in Down syndrome pregnancies. However, larger-scale studies are needed to validate these findings and explore the potential of combining multiple biomarkers to improve the accuracy of non-invasive prenatal testing. Such improvements could ultimately reduce the need for invasive diagnostic procedures and their associated risks, leading to better prenatal care outcomes.

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Author contributions

S. T. Project development, Data Collection, Manin Manuscript writing. A. O. Project development, Data Collection. F. Y. Project development, Data Collection. E. U. B. O. Project development. M. A. Data collection or management. C. G. O. S. Manuscript writing/editing. A. C. Manuscript writing/editing. All authors reviewed the manuscript.

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Data availability

The data that support the findings of this study are located in controlled access data storage at the Haseki Training and Research Hospital, Istanbul. Access is available upon written request to the authors.

Declarations

Ethical approval

This study was conducted in accordance with the latest Declaration of Helsinki after approval by the Clinical Research Ethics Committee of Haseki Training and Research Hospital, University of Health Sciences (Registration Number: 3-2024 dated January 23, 2024). The purpose and nature of all procedures performed were properly explained to each pregnant woman and she was asked to sign a written informed consent form to participate in this study.

Consent for publication

Not applicable. This manuscript does not contain any individual person's data in any form (including individual details, images, or videos).

Disclosure statement

During the preparation of this work, the authors used AI-assisted technologies (QuillBot AI) in order to improve readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Competing interests

The authors declare no competing interests.

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