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Unconjugated Estriol as a Marker for Adverse Pregnancy Outcome

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Abstract

Background: Unconjugated estriol (uE3) is an essential marker in prenatal biochemical screening for fetal health. Low uE3 levels are associated with several adverse outcomes.

Objectives: To investigate the associations between uE3 MoM ≤ 0.5 on second-trimester screening and adverse pregnancy outcomes.

Methods: The study used data from 5301 pregnant women referred to Nilou Laboratory in Tehran, Iran, during 2018 - 2021. Pregnancy and neonatal outcomes were evaluated according to uE3 MoM levels. The Mann–Whitney U test and Fisher's exact test were used for analysis.

Results: Associations were observed between uE3 MoM levels and several fetal and maternal complications. Greater nuchal translucency thickness (mean ± SD: 1.56 ± 0.41 vs. 1.51 ± 0.35), lower birth weight (mean ± SD: 3230 ± 1480 vs. 3248 ± 530.5), and lower birth height (mean ± SD: 50.24 ± 3.07 vs. 50.76 ± 8.19) were observed in the low-uE3 group (≤ 0.5 MoM; P = 0.02, 0.0008, and 0.03, respectively). The risk of cesarean delivery (relative risk = 1.28) and the frequency of consanguinity (relative risk = 1.25) were higher in this group (P < 0.001 and P = 0.0002, respectively). The risks of abortion (relative risk = 2.21) and maternal and neonatal intensive care unit admission (relative risks = 1.01 and 1.06, respectively) were also higher in this group (P < 0.001).

Conclusions: These findings are consistent with existing evidence showing associations between low uE3 MoM levels and adverse pregnancy outcomes. Low maternal uE3 MoM values may be associated with fetal pathology that warrants further investigation.

Keywords: Adverse Pregnancy Outcome, Unconjugated Estriol, Trisomy 18 Syndrome, Prenatal Screening, Biomarkers, Prenatal Diagnosis, Noninvasive Prenatal Testing

1. Background

Factors underlying adverse pregnancy outcomes have been repeatedly assessed in different populations (1-4). Unconjugated estriol (uE3) is an essential marker in prenatal biochemical screening for fetal health and well-being. Low uE3 levels are associated with Down syndrome, Edwards syndrome, Smith-Lemli-Opitz syndrome (SLOS), placental steroid sulfatase deficiency, and other adverse pregnancy outcomes. Therefore, this biomarker has long been used to diagnose these conditions (5, 6). Unconjugated estriol levels, expressed as multiples of the normal median (MoM), are used in second-trimester screening along with other markers, such as human chorionic gonadotropin, alpha-fetoprotein (AFP), and inhibin A (7, 8).

Unexplained abnormal uE3 MoM levels have also been associated with various adverse pregnancy outcomes. For

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instance, low uE3 MoM levels are associated with preterm birth, fetal growth restriction, low birth weight (LBW), and preeclampsia. Conversely, high uE3 MoM levels in the second trimester are not associated with adverse outcomes (9-11). Although uE3 may be considered a marker of fetal health, most studies have focused on other markers, including AFP and hCG. Few studies have investigated its prognostic value. In addition, the association between abnormal uE3 MoM levels and adverse pregnancy outcomes remains unclear. Several factors, including maternal weight, gestational age, and race, may influence uE3 concentrations (7, 12).

Given the importance of adverse pregnancy outcomes to the health care system (13), we previously demonstrated the value of free β-hCG MoM in identifying pregnancies at risk of adverse maternal and fetal outcomes, regardless of other abnormal first-trimester screening findings (14). Building on our previous studies evaluating the efficacy of first- and second-trimester biochemical markers and their relationships with chromosomal disorders, we compared uE3 MoM levels in relation to adverse maternal and fetal pregnancy outcomes.

2. Objectives

We aimed to investigate the associations between uE3 MoM ≤ 0.5 in second-trimester screening results and adverse pregnancy outcomes. Because fetal sex may affect both pregnancy outcomes and uE3 MoM levels, we also evaluated associations between fetal sex and these parameters.

Although low uE3 MoM levels have historically been associated with trisomies, open spina bifida, and SLOS in the prenatal screening literature, the current study was not designed to evaluate diagnostic or predictive performance for these specific conditions. Instead, we focused on associations between low uE3 levels (≤ 0.5 MoM) and a broader range of adverse pregnancy outcomes, including preterm birth, LBW, preeclampsia, and fetal death.

3. Methods

3.1. Study Design and Setting

This retrospective cohort study used data from pregnant women who underwent second-trimester biochemical screening at Nilou Laboratory in Tehran, Iran, between January 2018 and December 2021. All participants provided written informed consent, and the institutional ethics committee approved the study protocol.

3.2. Source Population

The source population comprised all women with singleton pregnancies referred to Nilou Laboratory for routine second-trimester maternal serum screening at 15 - 20 weeks of gestation. Nilou Laboratory is a large private referral center that primarily serves a low-risk population referred by community obstetricians, although a minority of referrals are for high-risk indications, such as advanced maternal age or abnormal first-trimester findings.

3.3. Participants and Sampling

All women with singleton pregnancies who underwent second-trimester maternal serum screening at 15 - 20 weeks of gestation during the study period were initially eligible. The sampling frame included all available records that met the basic criteria rather than a random sample, representing a consecutive or complete sampling approach using the laboratory database.

3.3.1. Inclusion Criteria

- Singleton pregnancy
- Gestational age between 14 and 20 weeks at blood sampling
- Available data on maternal age, weight, gestational age, and serum marker levels (AFP, hCG, and uE3)
- Completed follow-up of pregnancy outcomes via a telephone questionnaire 8 - 12 months after delivery

3.3.2. Exclusion Criteria

- Multiple gestation (twins or higher-order pregnancies)
- Missing or incomplete biochemical marker data
- Known fetal chromosomal anomalies diagnosed before screening (e.g., by chorionic villus sampling)
- Incomplete follow-up or loss to follow-up
- Refusal to provide informed consent
- Maternal age outside the range of 18 - 45 years
- Gestational age < 14 or > 20 weeks
- Preexisting maternal conditions affecting uE3 levels (e.g., adrenal hyperplasia or renal or liver disease)
- Use of medications that interfere with estriol metabolism (corticosteroids or antibiotics)
- Unknown or unrecorded fetal sex (based on study findings showing an association with fetal sex)

3.4. Final Sample Selection

Of the initial pool, 5301 pregnancies met all inclusion criteria and had complete screening and outcome data, forming the final analytic cohort. Participants were divided into two groups according to uE3 MoM levels:

Group 1: Low uE3 (≤ 0.5 MoM), 1593 pregnancies (30%)

Group 2: Normal uE3 (> 0.5 MoM), 3708 pregnancies (70%)

3.5. Data Collection

Biochemical measurements: Serum uE3 was measured by chemiluminescence immunoassay using a Siemens kit on the Immulite 2000 XP system. MoM values were calculated using laboratory-specific medians and corrected for maternal weight, diabetes status, smoking, and ethnicity.

Outcome data: Trained research staff blinded to uE3 MoM status contacted participants by telephone 8 - 12 months after their expected delivery date and used a standardized questionnaire to collect pregnancy and neonatal outcome data.

Verification: Outcomes reported by telephone were verified against hospital delivery records, neonatal intensive care unit (ICU) logs, and outpatient prenatal records; high agreement was reported.

3.6. Participant Selection and Follow-Up Pathway

The final analytic cohort comprised 5301 pregnancies with complete screening and outcome data. Serum uE3 was measured by chemiluminescence immunoassay using a Siemens kit on the Immulite 2000 XP system. Individual uE3 measurements were converted to MoM values using the most recently obtained medians from 10000 corresponding records at Nilou Laboratory. Second-trimester screening risk results were calculated using the LMS Alpha program, version 8 (http://www. 63 lmsalpha.com/). MoM values were corrected for maternal weight, diabetes status, smoking, and ethnicity. Participants were contacted by telephone 8 - 12 months after delivery to assess maternal and fetal outcomes. Results were compared between participants with uE3 MoM ≤ 0.5 and those with uE3 MoM > 0.5.

3.7. Outcomes and Outcome Ascertainment

Outcomes were categorized into four groups: 1) screening and diagnostic test abnormalities, 2) maternal pregnancy complications, 3) fetal complications, and 4) neonatal outcomes. The primary adverse pregnancy outcomes were abortion (spontaneous loss before 20 weeks), preterm birth (delivery before 37 weeks), LBW (< 2500 g), preeclampsia (new-onset hypertension ≥ 140/90 mmHg after 20 weeks with proteinuria ≥ 300 mg/24 h), and fetal death (intrauterine fetal demise at ≥ 20 weeks). Secondary outcomes included cesarean delivery, ICU admissions, and other complications listed in Table 1.

3.7.1. Outcome Ascertainment

All women were contacted by telephone 8 - 12 months after their expected delivery date. Trained research staff who were blinded to uE3 MoM status administered a standardized questionnaire that collected data on:

- Gestational age at delivery
- Birth weight and height
- Mode of delivery (cesarean or vaginal)
- Pregnancy outcome (live birth, abortion, or fetal death)
- Maternal complications (hypertension, preeclampsia, gestational diabetes, hemorrhage, or intensive care unit (ICU) admission)
- Fetal and neonatal complications (structural anomalies, chromosomal abnormalities, respiratory problems, or neonatal ICU admission)

3.7.2. Verification Against Medical Records

Outcomes reported during telephone interviews were verified against hospital delivery records, neonatal ICU logs, and outpatient prenatal records. Agreement between telephone reports and medical records was high. All reported cases of infrequent outcomes (e.g., Turner syndrome and placenta percreta) were verified against medical records.

3.8. Statistical Analysis

To investigate possible associations with uE3 MoM levels, pregnancy characteristics were summarized using means, SDs, medians, and interquartile ranges (25th–75th percentiles) for continuous variables and frequencies for categorical variables. Statistical analyses were primarily exploratory and based on unadjusted comparisons because the study included multiple outcome groups with different denominators, substantial outcome-specific missing data, and several infrequent events. The uE3 MoM values had already been corrected for maternal weight, diabetes status, smoking, and ethnicity as part of the routine screening algorithm. Continuous variables were compared using the Mann–Whitney U test, and categorical variables were compared using Fisher's exact test. The number of participants analyzed for each outcome is reported separately according to data availability. Statistical analyses were performed using GraphPad Prism, version 9.0 (GraphPad Software, La Jolla, CA, USA). Statistical significance was defined as $P < 0.05$.

4. Results

Table 1. Mean, Standard Deviation, Median and Quartiles of Continuous Variables Data in Two Groups of Patients Based on the Levels of uE3 MoM (uE3 MoM > 0.5 (N = 3708) vs. ≤ 0.5 (N = 1593)) [a]

Variables (Continuous data)	Low uE3 MoM (≤0.5) Levels (n = 1593) (30%)	Normal uE3 MoM (>0.5) Levels (n = 3708) (70%)	P Value [b]
Maternal age at expected delivery date (y)	30.2 ± 4.8, 30.5 (26.9, 34)	30.3 ± 5.5, 30.6 (26.3, 34.2)	0.97
Gravidity	2.08 ± 1.17, 2 (1, 3)	1.87 ± 1.05, 2 (1, 2)	< 0.001
Parity	0.78 ± 0.85, 1 (0, 1)	0.59 ± 0.76, 0 (0, 1)	< 0.001
Gestational age at delivery (weeks)	38.21 ± 2.21, 38 (38, 39)	38.35 ± 1.88, 38 (38, 40)	0.065
Nuchal translucency (mm)	1.56 ± 0.41, 1.5 (1.3, 1.72)	1.51 ± 0.35, 1.5 (1.3, 1.73)	0.02
Crown-rump length (mm)	59.18 ± 8.84, 59 (54, 64)	58.79 ± 8.54, 58 (53.75, 63.06)	0.207
Birth weight (g)	3230 ± 1480, 3200 (2900, 3500)	3248 ± 530.5, 3250 (2970, 3550)	< 0.001
Birth height (cm)	50.24 ± 3.07, 50 (49, 52)	50.76 ± 8.19, 50 (49, 52)	0.03

[a] Values are expressed as mean ± SD, median (1st, 3rd quartiles).

[b] Mann–Whitney U test

Table 1 compares continuous demographic, anthropometric, and pregnancy-related variables between the two study groups, defined by uE3 MoM levels. A total of 5301 pregnancies were analyzed, including 1593 (30%) in the low-uE3 group (≤ 0.5 MoM) and 3708 (70%) in the normal-uE3 group (> 0.5 MoM). Continuous variables were compared using the Mann–Whitney U test because the data were not normally distributed.

Among the variables assessed, nuchal translucency thickness was significantly greater in the low-uE3 group than in the normal-uE3 group (mean ± SD: 1.56 ± 0.41 mm vs. 1.51 ± 0.35 mm; P = 0.02), suggesting an association between low uE3 levels and increased first-trimester markers of aneuploidy risk. Birth weight was significantly lower in the low-uE3 group (3230 ± 1480 g vs. 3248 ± 530.5 g; P < 0.001), as was birth height (50.24 ± 3.07 cm vs. 50.76 ± 8.19 cm; P = 0.03), indicating impaired fetal growth parameters in pregnancies with low uE3 levels.

In contrast, no significant differences were observed between the two groups in maternal age at the expected delivery date (30.2 ± 4.8 vs. 30.3 ± 5.5 years; P = 0.97), gestational age at delivery (38.21 ± 2.21 vs. 38.35 ± 1.88 weeks; P = 0.065), or crown-rump length (59.18 ± 8.84 vs. 58.79 ± 8.54 mm; P = 0.207). These findings suggest that the observed differences are specific to fetal growth and screening markers rather than to general maternal or pregnancy characteristics.

Table 2 summarizes the frequencies and relative risks of categorical variables related to maternal history, pregnancy characteristics, and neonatal outcomes in the two groups. Fisher's exact test was used to compare categorical data.

The proportion of male fetuses was significantly higher in the low-uE3 group than in the normal-uE3 group (59.33% vs. 47.97%; relative risk [RR] = 1.23; P < 0.001), indicating a strong association between low uE3 levels and male fetal sex. The cesarean delivery rate was also significantly higher in the low-uE3 group (76.1% vs. 69.47%; RR = 1.28; P < 0.001), suggesting that pregnancies with low uE3 levels are more likely to require surgical intervention. Consanguineous marriages were also more frequent in the low-uE3 group (22.56% vs. 18.06%; RR = 1.25; P < 0.001), suggesting a possible genetic contribution to low uE3 levels.

Regarding pregnancy outcomes, the risk of abortion was more than twofold higher in the low-uE3 group (8.85% vs. 3.99%; RR = 2.21; P < 0.001), highlighting the association between low uE3 levels and early pregnancy loss. Maternal ICU admission (2.2% vs. 0.74%; RR = 1.01; P < 0.001) and neonatal ICU admission (12.3% vs. 7.25%; RR = 1.06; P < 0.001) were both significantly more frequent in the low-uE3 group, indicating increased maternal and neonatal morbidity. The frequency of problems at birth was lower in the low-uE3 group (15.84% vs. 20.31%; RR = 0.78; P = 0.001). This may be explained by the higher abortion rate in this group, as pregnancies with severe problems may have been terminated before birth.

In contrast, a history of IVF, a history of diabetes, and smoking were observed exclusively in the normal-uE3 group, and all three differences were statistically significant (P < 0.001). Preterm labor (9.7% in both groups; P > 0.99) and a history of Down syndrome (0% vs. 0.08%; P = 0.559) did not differ significantly between the two groups.

Table 3 compares the frequencies of abnormal prenatal screening and diagnostic test findings between the two groups. Fisher's exact test was used for statistical analysis. Abnormal amniocentesis results were observed only in the low-uE3 group, with a frequency of 34%, compared with 0% in the normal-uE3 group (P < 0.001). This strong association suggests that low uE3 levels are a powerful indicator of fetal chromosomal abnormalities warranting invasive diagnostic testing. However, this association was observed

Table 2. Maternal and Neonatal Characteristics and Outcomes in the Low and Normal uE3 MoM Groups [a]

Variables	Low uE3 (≤ 0.5 MoM; n = 1593 [30%])	Normal uE3 (> 0.5 MoM; n = 3708 [70%])	P Value [b]
Preterm labor (before 37 weeks of gestation)	134 (9.7), 0.99	282 (9.7)	> 0.99
Fetal sex (male)	906 (59.33), 1.23	1488 (47.97)	< 0.001
Mode of delivery			< 0.001
Cesarean delivery	1110 (76.1), 1.28	2082 (69.47), 1	
Normal vaginal delivery	348 (23.87), 0.78	915 (30.53), 1	
Previous pregnancies			< 0.001
No	663 (42.58), 0.82	1929 (53.1), 1	
Yes	894 (57.42), 1.22	1704 (46.9), 1	
Previous abortions			0.050
No	1155 (74.32), 0.9	2787 (76.78), 1	
Yes	399 (25.68), 1.1	843 (23.22), 1	
Consanguinity			< 0.001
Consanguineous	354 (22.56), 1.25	669 (18.06), 1	
Nonconsanguineous	1215 (77.44), 0.8	3036 (81.94), 1	
History of Down syndrome	0 (0), 0	3 (0.08), 1	0.559
History of IVF	0 (0), 0	39 (1.05), 1	< 0.001
History of diabetes	0 (0), 0	27 (0.72), 1	< 0.001
Smoking	0 (0), 0	42 (1.1), 1	< 0.001
Pregnancy outcome			< 0.001
Live birth	1452 (91.15), 0.45	3537 (96.01), 1	
Abortion	141 (8.85), 2.21	147 (3.99), 1	
Maternal and neonatal outcomes following live birth			
Early discharge after birth	1239 (85.5), 0.98	2586 (91.9), 1	< 0.001
Maternal admission to the intensive care unit	33 (2.2), 1.01	21 (0.74), 1	< 0.001
Admission to the neonatal intensive care unit	180 (12.3), 1.06	204 (7.25), 1	< 0.001
Problems at birth			0.001
No	1211 (84.11), 1.28	1389 (79.69), 1	
Yes	228 (15.84), 0.78	354 (20.31), 1	

[a] Values are expressed as No. (%), RR. Percentages are calculated using the total number of participants in each uE3 group as the denominator. Abbreviation: RR, relative risk.

[b] Fisher's exact test.

Table 3. Frequencies of Abnormal screening/diagnostic Tests in Two Groups of Patients Based on the Levels of uE3 MoM (>0.5 and ≤ 0.5) [a]

Study Variables (Categorical Data)	Low uE3 MoM (≤ 0.5) Levels (n = 1593) (30%)	Normal uE3 MoM (> 0.5) Levels (n = 3708) (70%)	P Value
Abnormal maternal sonography	9 (0.61), 0.07	291 (8.05), 1	< 0.001
Abnormal placenta sonography	81 (5.5), 0.43	537 (12.58), 1	< 0.001
Abnormal fetal sonography	9 (0.61), 1.47	15 (0.42), 1	0.37
Abnormal fetal echocardiography	12 (1.7), 1.02	24 (1.66), 1	> 0.99
Abnormal cell free fetal DNA	0 (0)	0 (0)	> 0.99
Abnormal amniocentesis	51 (34), Not estimated [b]	0 (0), 1 [b]	< 0.001
Abnormal Anomaly scan	39 (3.5), 5.18	12 (0.68), 1	< 0.001

[a] Values are expressed as No. (%), relative risks. Abbreviations: RR, relative risk.

[b] For amniocentesis, percentages were calculated among women with a recorded amniocentesis result rather than among the total uE3-defined group. The denominator was 150 in the low-uE3 group and 69 in the normal-uE3 group.

among women undergoing invasive diagnostic testing and should not be interpreted as evidence that low uE3 levels

alone constitute a diagnostic or predictive test for fetal chromosomal abnormalities.

Similarly, abnormal anomaly scan findings were significantly more frequent in the low-uE3 group (3.5% vs. 0.68%; RR = 5.18; P < 0.001), indicating a more than fivefold increase in the risk of structural fetal anomalies in pregnancies with low uE3 levels.

Conversely, abnormal maternal ultrasonography findings (0.61% vs. 8.05%; RR = 0.07; P < 0.001) and abnormal placental ultrasonography findings (5.5% vs. 12.58%; RR = 0.43; P < 0.001) were significantly less frequent in the low-uE3 group. These seemingly paradoxical findings may be due to selection bias, as pregnancies with obvious ultrasonographic abnormalities might have undergone direct invasive testing (amniocentesis) without being included in the routine ultrasonography categories. Alternatively, these findings could reflect differences in referral patterns between the two groups.

No significant differences were observed between the two groups in abnormal fetal ultrasonography findings (0.61% vs. 0.42%; P = 0.37), abnormal fetal echocardiography findings (1.7% vs. 1.66%; P > 0.99), or abnormal cell-free fetal DNA test results (0% in both groups; P > 0.99), suggesting that these tests are not differentially affected by uE3 MoM levels.

Table 4 compares the frequencies of common pregnancy complications, maternal diseases, adverse maternal outcomes, and adverse fetal outcomes between the two groups. Fisher's exact test was used for all statistical comparisons. The table is organized into four sections. Among common pregnancy complications, hypertension was significantly more frequent in the low-uE3 group (11.7% vs. 9.58%; RR = 1.22; P = 0.02), as were proteinuria (8.58% vs. 6.42%; RR = 1.33; P = 0.008) and vaginal bleeding (4.48% vs. 2.89%; RR = 1.55; P = 0.005). These findings suggest an increased risk of preeclampsia and placental dysfunction in pregnancies with low uE3 levels. Vaginal discharge was less frequent in the low-uE3 group (0.97% vs. 1.78%; RR = 0.54; P = 0.039). No significant differences were observed in edema, premature rupture of membranes, or light bleeding (spotting).

Regarding maternal diseases, anemia was more common in the low-uE3 group (22.22% vs. 1.55%; RR = 14.3; P < 0.001), representing a 14-fold increase in risk. Epilepsy was also significantly more frequent (5.56% vs. 0.52%; RR = 10.72; P = 0.009), as were other diseases (27.78% vs. 4.66%; RR = 5.95; P < 0.001). In contrast, hypothyroidism was significantly more common in the normal-uE3 group (38.89% vs. 87.56%; RR = 0.44; P < 0.001). This may reflect the higher prevalence of thyroid disorders in the larger normal-uE3 group or the possible protective effects of low uE3 levels on thyroid function. No significant difference in diabetes was observed between the two groups.

Among adverse maternal outcomes, abortion was significantly more frequent in the low-uE3 group (13.62% vs. 5.75%; RR = 2.36; P < 0.001), consistent with the findings in Table 2. Maternal infection (3.29% vs. 1.5%; RR = 2.19; P = 0.01), severe hemorrhage during pregnancy (6.1% vs. 2.25%; RR = 2.71; P < 0.001), pregnancy-induced hypertension (15.49% vs. 11.75%; RR = 1.31; P = 0.024), placenta percreta (0.47% vs. 0%; RR = infinity; P = 0.04), and preeclampsia (0.47% vs. 0%; RR = infinity; P = 0.04) were all significantly associated with low uE3 levels. Conversely, premature rupture of membranes (30.52% vs. 37%; RR = 0.82; P = 0.0055), hematoma behind the placenta (0% vs. 5.75%; RR = 0; P < 0.001), amniotic fluid leakage (0.47% vs. 3%; RR = 0.15; P < 0.001), and oligohydramnios (2.35% vs. 4.25%; RR = 0.55; P = 0.03) were less frequent in the low-uE3 group. No significant differences were observed in gestational diabetes mellitus, polyhydramnios, HELLP syndrome, spotting, or severe hemorrhage after delivery.

Adverse fetal outcomes showed the strongest associations with low uE3 MoM levels. Trisomy 18 (3.31% vs. 0%; RR = infinity; P < 0.001), intrauterine fetal death (1.65% vs. 0%; RR = infinity; P = 0.001), Turner syndrome (0.83% vs. 0%; RR = infinity; P = 0.04), choroid plexus cyst (1.65% vs. 0%; RR = infinity; P = 0.04), hypoplastic nasal bone (0.83% vs. 0%; RR = infinity; P = 0.04), absent nasal bone (0.83% vs. 0%; RR = infinity; P = 0.04), and skeletal dysplasia (0.83% vs. 0%; RR = infinity; P = 0.04) were observed exclusively in the low-uE3 group. These infinite relative risk estimates reflect the occurrence of these conditions only in pregnancies with low uE3 levels and their absence from the normal-uE3 group, suggesting a very strong association.

Structural anomalies (13.22% vs. 5.45%; RR = 2.42; P < 0.001), metabolic disorders (2.48% vs. 0.45%; RR = 5.45; P = 0.01), LBW (1.65% vs. 0.91%; RR = 1.8; P < 0.001), and other chromosomal abnormalities (0.83% vs. 0%; RR = infinity; P = 0.04) were also significantly more frequent in the low-uE3 group. Respiratory problems were more common in the low-uE3 group (13.22% vs. 9.09%; RR = 1.45; P = 0.04). In contrast, neonatal hospitalization was less frequent in the low-uE3 group (13.22% vs. 23.64%; RR = 0.55; P < 0.001). This may be explained by the higher rates of abortion and intrauterine fetal death in this group, resulting in fewer live-born neonates requiring hospitalization.

No significant differences were observed between the two groups in trisomy 21 (Down syndrome), imperforate anus, fetal morbidity, echogenic cardiac focus, neural tube defects, death after delivery, hydrocephaly, hydrops fetalis, nuchal cord, or intrauterine growth restriction.

5. Discussion

We evaluated pregnancy and neonatal outcomes in a large cohort according to uE3 MoM levels and identified associations between this marker and several fetal and maternal complications. nuchal translucency thickness was greater, and birth weight and height were lower, in the low-uE3 group (≤ 0.5 MoM). The risk of cesarean delivery and the frequency of consanguinity were also higher in this group. Moreover, the risks of abortion and of maternal and neonatal ICU admission were higher. A previous study identified 62 women with very low uE3 MoM levels among 44,146 pregnancies screened. Most of these cases were associated with fetal death, and some involved placental sulfatase deficiency. The authors therefore suggested that very low second-trimester uE3 MoM levels were associated with an increased likelihood of early fetal demise and placental sulfatase deficiency (15). They also reported a higher proportion of male infants among patients with unexplained very low uE3 MoM levels (15), consistent with the current findings. Another study of more than 130,000 pregnancies identified undetectably low uE3 MoM levels in 167 cases. The most common explanations were fetal death, overestimation of gestational age, nonpregnancy, and chromosomal abnormalities (16). Consistent with the earlier report (15), the authors identified an association between unexplained low uE3 MoM levels and male fetal sex (16).

Another study associated low uE3 MoM levels with dating errors, prematurity, growth restriction, and X-linked ichthyosis (17). A more recent study reported an association between low uE3 MoM levels and the likelihood of fetal growth restriction and LBW, but not preterm birth or other adverse outcomes (18). In the current study, birth weight was lower in pregnancies with low uE3 MoM levels. However, the risk of LBW (< 2500 g) did not differ between the two groups.

Another study found that women with false-positive screening results for trisomy 21 had higher risks of preterm delivery, preeclampsia, small-for-gestational-age birth, and fetal death. Notably, uE3 MoM values of 0.75 or less were associated with adverse pregnancy outcomes after adjustment for MSAFP and hCG (19). Low uE3 levels have also been associated with placenta-mediated complications, including preeclampsia, fetal growth restriction, placental abruption, stillbirth, and preterm birth (20). The current study also confirmed associations between low uE3 MoM levels and preeclampsia and fetal death.

The results of this study are consistent with the body of evidence showing associations between low uE3 MoM levels and adverse pregnancy outcomes. Our findings suggest that low maternal uE3 MoM values may be associated with fetal pathology warranting further investigation. However, given the observational design, potential residual confounding, loss to follow-up, and exploratory nature of the multiple secondary comparisons, these findings should be interpreted as hypothesis-generating rather than definitive. Low uE3 MoM appears to be a marker of elevated risk rather than a direct cause of adverse outcomes. The clinical utility of isolated low uE3 MoM for predicting specific complications remains uncertain in the absence of confirmatory testing and integration with other biomarkers.

We recommend enhanced antenatal surveillance, including serial growth scans and blood pressure monitoring, for pregnancies with unexplained low uE3 levels (≤ 0.5 MoM), while acknowledging that most such pregnancies will have normal outcomes. Future prospective, multicenter studies with standardized outcome definitions, verification against medical records, and adjustment for a comprehensive set of confounders are needed to validate these associations and determine whether low uE3 MoM improves risk prediction beyond established clinical factors.

5.1. Study Limitations

This study has several limitations. First, the retrospective observational design limits causal interpretation; therefore, we report associations only. Findings from this single-center referral laboratory population may not be generalizable to broader or more diverse prenatal cohorts. Outcome ascertainment by telephone introduces potential selection and recall biases. Outcome-specific missing data and varying denominators limited the feasibility of conducting a uniformly adjusted multivariable analysis across all outcomes. Several outcomes were rare, with fewer than 5 events per group for conditions such as Turner syndrome (n = 3), placenta percreta (n = 3), and skeletal dysplasia (n = 3). This resulted in unstable relative risk estimates and an increased risk of type I error in the setting of multiple comparisons. Despite adjustment for several screening-related factors (maternal weight, diabetes status, smoking, and ethnicity) during MoM calculation, residual confounding from unmeasured variables, such as maternal stress, nutritional status, environmental exposures, or genetic predispositions, cannot be excluded. Finally, we did not analyze diagnostic performance for specific conditions, such as trisomies, open spina bifida, or SLOS; therefore, no predictive claims can be made for these disorders. External validation in independent, population-based cohorts is needed. Although uE3 MoM values were corrected for several screening-related factors, residual confounding cannot be excluded. The findings should therefore be interpreted as exploratory associations rather than definitive causal

effects. Given the observational design, the large number of comparisons, and the exploratory nature of this study, all findings should be considered hypothesis-generating rather than confirmatory or definitive.

Footnotes

AI Use Disclosure: DeepSeek was used for text editing in the Results section at a moderate level.

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Data Availability: The data sets generated and analyzed during the study are available from the corresponding author upon reasonable request.

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Table 4. Pregnancy Complications, Maternal Diseases, and Adverse Maternal and Fetal Outcomes in the Low and Normal uE3 MoM Groups [a]

Variables	Low uE3 (≤ 0.5 MoM; n = 1593 [30%])	Normal uE3 (> 0.5 MoM; n = 3708 [70%])	P Value
Common pregnancy complications			
Hypertension	180 (11.7), 1.22	291 (9.58), 1	0.02
Proteinuria	132 (8.58), 1.33	195 (6.42), 1	0.008
Edema	249 (16.18), 1.06	462 (15.2), 1	0.38
Vaginal bleeding	69 (4.48), 1.55	87 (2.89), 1	0.005
Premature rupture of membranes	222 (14.42), 0.88	498 (16.39), 1	0.08
Light bleeding (spotting)	147 (9.55), 0.96	300 (9.87), 1	0.73
Vaginal discharge	15 (0.97), 0.54	54 (1.78), 1	0.039
Maternal diseases			
Diabetes	3 (5.56), 0.97	33 (5.7), 1	0.15
Anemia	12 (22.22), 14.3	9 (1.55), 1	< 0.001
Hypothyroidism	21 (38.89), 0.44	507 (87.56), 1	< 0.001
Epilepsy	3 (5.56), 10.72	3 (0.52), 1	0.009
Other diseases	15 (27.78), 5.95	27 (4.66), 1	< 0.001
Total	54 (3.39), 0.21	579 (15.59), 1	< 0.001
Adverse maternal outcomes			
Gestational diabetes mellitus	18 (2.82), 1.2	27 (2.25), 1	0.52
Preterm delivery	21 (3.29), 0.62	63 (5.25), 1	0.06
Premature rupture of membranes	195 (30.52), 0.82	444 (37), 1	0.0055
Abortion	87 (13.62), 2.36	69 (5.75), 1	< 0.001
Polyhydramnios	0 (0), 0	3 (0.25), 1	0.56
Maternal infection	21 (3.29), 2.19	18 (1.5), 1	0.01
Hematoma behind the placenta	0 (0), 0	69 (5.75)	< 0.001
Severe hemorrhage during pregnancy	39 (6.1), 2.71	27 (2.25), 1	< 0.001
HELLP syndrome	3 (0.47), 0.46	12 (1), 1	0.72
Pregnancy-induced hypertension	99 (15.49), 1.31	141 (11.75), 1	0.024
Amniotic fluid leakage	3 (0.47), 0.15	36 (3), 1	< 0.001
Spotting	78 (12.2), 0.88	165 (13.75), 1	0.73
Severe hemorrhage after delivery	3 (0.47), 1.87	3 (0.25), 1	0.38
Placenta percreta	15 (2.35), 0.55	51 (4.25), 1	0.03
Oligohydramnios	3 (0.47), infinity	0 (0), 1	0.04
Preeclampsia	54 (8.45), 1.4	72 (6), 1	0.05
Total	639 (40.11), 1.24	1200 (32.31), 1	< 0.001
Adverse fetal outcomes			
Trisomy 21	36 (9.92), 0.8	81 (12.27), 1	0.30
Imperforate anus	0 (0), 0	6 (0.91), 1	0.095
Fetal morbidity	87 (23.97), 1.01	156 (23.64), 1	0.93
Premature rupture of membranes	0 (0), 0	18 (2.73), 1	< 0.001
Trisomy 18	12 (3.31), infinity	0 (0), 1	< 0.001
Echogenic cardiac focus	0 (0), 0	6 (0.91), 1	0.095
Respiratory problems	48 (13.22), 1.45	60 (9.09), 1	0.04
Neural tube defects	6 (1.65), 3.6	3 (0.45), 1	0.075
Death after delivery	0 (0), 0	3 (0.45), 1	0.55
Hydrocephaly	0 (0), 0	3 (0.45), 1	0.55
Metabolic disorders	9 (2.48), 5.45	3 (0.45), 1	0.01
Neonatal hospitalization	48 (13.22), 0.55	156 (23.64), 1	< 0.001
Intrauterine fetal death	6 (1.65), infinity	0 (0), 1	0.001
Structural anomalies	48 (13.22), 2.42	36 (5.45), 1	< 0.001
Hematoma behind the placenta	0 (0), 0	3 (0.45), 1	0.55

Variables	Low uE3 (≤ 0.5 MoM; n = 1593 [30%])	Normal uE3 (> 0.5 MoM; n = 3708 [70%])	P Value
Turner syndrome	3 (0.83), infinity	0 (0), 1	0.04
Death due to structural anomalies	24 (6.61), 2.42	18 (2.73), 1	0.004
Choroid plexus cyst	0 (0), 0	12 (1.82), 1	0.006
Hypoplastic nasal bone	3 (0.83), 0.08	63 (9.55), 1	< 0.001
Skeletal dysplasia	0 (0), 0	3 (0.45), 1	0.095
Absent nasal bone	0 (0), 0	6 (0.91), 1	0.36
Low birth weight	6 (1.65), 1.8	6 (0.91), 1	< 0.001
Hydrops fetalis	3 (0.83), infinity	0 (0), 1	0.04
Other chromosomal abnormalities	0 (0), 0	3 (0.45), 1	0.55
Nuchal cord	3 (0.83), infinity	0 (0), 1	0.04
Intrauterine growth restriction	3 (0.83), infinity	0 (0), 1	0.04
Placenta percreta	3 (0.83), infinity	0 (0), 1	0.04
Oligohydramnios	3 (0.83), infinity	0 (0), 1	0.04
Total	363 (22.79), 1.28	660 (17.77), 1	< 0.001

[a] Values are expressed as No. (%), RR. The adverse maternal outcome analysis includes participants with available data for this outcome category. Percentages in the adverse maternal outcomes section are calculated using participants with available data for this category (n = 639 in the low-uE3 group and n = 1200 in the normal-uE3 group), rather than the total number of participants in each uE3-defined group. Abbreviation: RR, relative risk.