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Diagnostic value of CA 19-9 in pregnancies complicated by spinal neural tube defects: a preliminary study

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ABSTRACT

Objectives: Various physiological and pathological conditions can induce significant variations in plasma concentrations of tumor markers, such as CA 19-9, which is present in the serum and amniotic fluid of pregnant women. Herein, we aimed to determine the clinical importance of maternal serum CA 19-9 levels in the diagnosis of neural tube defects (NTDs).

Material and methods: A total of 100 women were included in this controlled cross-sectional study. Thirty-three patients whose pregnancies were complicated by isolated meningocele or meningomyelocele constituted the study group, whereas 33 normal, healthy pregnant women constituted the control group, and 34 age- and body mass index (BMI)-matched non-pregnant women were chosen for the validation group.

Results: The mean maternal serum CA 19-9 levels were 17.2 ± 17.0 IU/mL, 7.1 ± 5.9 IU/mL, and 4.7 ± 3.6 IU/mL in the study, control, and validation groups, respectively ($p < 0.001$). ROC analyses showed that elevated CA 19-9 values may predict NTDs ($p < 0.001$). The cut-off value for CA 19-9 was found to be 9.6 IU/mL at 70% (51%–84%, 95% CI) sensitivity and 84% (74%–92%, 95% CI) specificity.

Conclusions: CA 19-9 may be a promising noninvasive marker for NTDs. Further studies are needed to reveal the clinical applicability and diagnostic potential of maternal serum CA 19-9 levels in the identification of NTDs.

Key words: inflammation, meningomyelocele, prenatal screening, spina bifida, tumor markers

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INTRODUCTION

Neural tube defect (NTD) is the second most common cause of congenital anomalies, after congenital heart disease. The etiology of NTDs has been extensively studied and it is believed that they are multifactorial complex disorders. Genetic predisposition, socioeconomic status, diabetes mellitus, and environmental causes, such as nutritional deficiency, anticonvulsant agents, and hyperthermia, may affect the risk for NTDs [1, 2]. The reported incidence of NTDs (of which myelomeningocele is the most common) varies between different geographic regions; the rate has been reported to be as low as 1 per 1,000 live births in European countries, and as high as 15 per 1,000 live births in some Asian countries, such as India [3]. NTDs originate from the failure of the neurulation process during embryonic development, 3–4 weeks after conception [4]. These defects are divided into two types: cranial and spinal. Encephalocele, anencephaly, and iniencephaly are lethal cranial defects, while meningocele and meningomyelocele are open spinal NTDs, and spina bifida occulta is a closed spinal NTD [5]. The exact cause of NTDs is not known, but a diet poor in folic acid increases the risk; folic acid supplementation is known to decrease the rate of NTDs by 27–40% [6]. NTDs are generally diagnosed via imaging methods, and these pregnancies are often terminated.

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Carbohydrate antigen 19-9 (CA 19-9), also called sialylated Lewis antigen, is synthesized by normal human pancreatic and biliary ductal cells and by gastric, colon, endometrial, and salivary excretory epithelial tissues [7, 8]. CA 19-9 is commonly used as a tumor marker for certain epithelial-type gastrointestinal cancers, particularly pancreatic. However, elevated serum levels of CA 19-9 can be detected in several benign pancreatic, hepatic, and biliary conditions, such as acute and chronic pancreatitis, cholelithiasis, cholecystitis, achalasia, acute hepatitis, and hepatic cirrhosis, and in benign lung diseases, such as bronchiolitis, emphysema, and bronchiectasia [9, 10]. Systemic diseases, such as diabetes mellitus, rheumatic disorders, and autoimmune disorders, may also increase CA 19-9 serum levels. In addition, it is used as a follow-up marker for chronic organ-specific inflammatory conditions, such as prostatitis [11].

The idea of prenatal screening for serious birth defects began in the 1970s with the discovery of high maternal serum levels of alpha-fetoprotein (AFP) in pregnancies complicated by open NTDs. It was hypothesized that AFP, one of several oncofetal proteins synthesized in large amounts by the fetal liver, leaks into the amniotic fluid from the open side of the central nervous system, and leads to high maternal serum AFP levels. Similarly, the oncofetal antigen CA 19-9 may diffuse to the maternal circulation through the amniotic fluid in open NTDs. Previous experimental studies in animals showed that exposure of the spinal cord to amniotic fluid contributes to local tissue inflammation and associated neurological sequelae [12, 13]. Neuronal inflammation can also lead to elevated serum CA 19-9 levels [14].

Neutrophil and lymphocyte cells play an important role in inflammatory conditions, and their numbers and structures may change in case of inflammation. The neutrophil-to-lymphocyte ratio (NLR) is a simple biomarker for inflammation; in a recent study, NLR was introduced as a marker in various diagnostic and prognostic gynecological procedures [15]. NLR has been also studied in some acute and chronic inflammatory diseases of the central nervous system. It was suggested as a potential biomarker in the differential diagnosis of acute meningitis [16], and as a predictor of disease activity and severity in multiple sclerosis [17]. In the present study, we aimed to investigate serum CA 19-9 levels in pregnancies with spinal NTDs.

MATERIAL AND METHODS

This prospective, cross-sectional case-control study was conducted at the Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, a referral tertiary hospital located in the central region of Turkey, with approximately 20,000 deliveries per year. A total of 100 pregnant and non-pregnant women were recruited from high-risk pregnancy, antenatal, and family planning clinics between January 2015 and August 2015, after the study received approval from the hospital's ethics committee. Informed consent was obtained from each participant. *All of the patients were in the second trimester of pregnancy (18–22 weeks), and each* underwent a detailed prenatal ultrasound screening with a Voluson 730 Expert ultrasound machine (GE Medical systems, Kretztechnik GmbH & OHG, Zipf, Austria) equipped with a 3.5–5-MHz curvilinear transabdominal transducer. Ultrasonographic examinations were performed by a high-risk-pregnancy specialist in the antenatal polyclinic. Thirty-three patients whose pregnancies were complicated by isolated meningocele or meningomyelocele constituted the study group (group I), whereas 33 normal, healthy pregnant women constituted the control group (group II) and 34 age- and body mass index (BMI)-matched non-pregnant women, who had given birth at least 6 weeks earlier and had applied for birth control methods, were chosen for the validation group (group III). None of the patients in the validation group had not been used a birth control method yet. Most of the patients with NTDs were referred by other hospitals in the region. The exclusion criteria were a known history or evidence of acute infection; chronic inflammatory disease; respiratory, rheumatologic, or immunologic disease; pancreatic, hepatic, or biliary tract disease; gestational diabetes; hypertensive disorders; a previous history of ovarian cysts; or any malignancy. It was confirmed that postpartum or postabortion examination of the fetuses showed no additional anomalies in the study group. Patients with a suspected diagnosis of NTD in the detailed ultrasound scan were excluded from the study. All patients were also evaluated during the ultrasound examination for the presence of any adnexal masses, and no such masses or cysts were observed.

The data obtained from the patients were age, BMI, gestational age at blood draw in the pregnant patients, obstetrical history (gravidity, parity, and number of live children and abortions), and smoking status. Blood samples were taken from pregnant women at the time of applying for ultrasound examination whereas they were collected from non-pregnant women during the early follicular phase (day 2–4). The patients' height and weight were measured with a professional calibrated device. BMI was calculated based on the following formula: weight (kg)/height (m^2). Gestational weeks were calculated according to last menstrual period, or with ultrasound during the first trimester in patients who did not know their last menstrual period.

Following at least 8 hours of fasting, approximately 5 cc of blood was drawn from the antecubital vein and poured into gel tubes and hematologic sample tubes containing anticoagulant. The blood samples were centrifuged at 4100 × g for 10 minutes. The sera were then separated, and were studied immediately. Serum CA 19-9 levels were measured

with an Analytics E170 (Elecsys module) immunoassay analyzer (Roche Diagnostics GmbH, Mannheim, Germany) using the electrochemiluminescence immunoassay method. Hematologic parameters were investigated using the Coulter LH–780 hematology blood analyzer (Beckman Coulter Inc., Brea, CA, USA). NLR was defined as absolute neutrophil count divided by absolute lymphocyte count. All blood samples were analyzed in the biochemistry laboratory of our hospital.

Statistical analysis

Statistical Package for the Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were used to demonstrate the mean ± standard deviation and the median (range) for constant variables, whereas nominal variables were expressed as case numbers and percentages. For the analysis of the data, normality in the repeated measures was tested with the Kolmogorov-Smirnov or Shapiro-Wilk tests. One-way ANOVA was performed for data that demonstrated a normal distribution, and the Mann-Whitney U test and the Kruskal-Wallis test were used for data that did not demonstrate a normal distribution. Homogeneity of the variances was evaluated with Levene's test. Tukey's honest significant difference (HSD) post-hoc test was used when the homogeneity requirement was met, and the Games Howell test was used otherwise. Nominal variables were analyzed with a chi-square test. In order to generate a cut-off value for CA 19-9, a receiver operating characteristic (ROC) curve analysis was used. To determine the cut-off values corresponding to the CA 19-9, the Youden index (sensitivity + specificity – 1) was used, and the highest Youden index value was considered the cut-off. Pearson's correlation analysis was used to determine the relationship between CA 19-9 and NLR. Statistical significance was accepted as $p < 0.05$.

RESULTS

A total of 100 women were included in this study. Thirty-three pregnant women whose pregnancies were complicated by spinal NTDs were classified as group I, 33 healthy pregnant women without fetal malformations were classified as group II, and group III consisted of 34 healthy non-pregnant women. Of the 33 patients in group I, 10 had meningocele and 23 had meningomyelocele. Table 1 compares the demographic characteristics of the groups. All three groups were similar in terms of age and BMI. Median parity and the number of live children were significantly higher in the validation group compared with the other two groups ($p < 0.05$). The rate of current smokers was also higher in this group, but the difference was statistically insignificant. There was no statistically significant difference between the pregnant groups regarding fetal gender and gestational age at blood draw (19.9 ± 2.2 weeks *vs.* 20.1 ± 1.5 weeks, respectively; $p = 0.163$).

Twenty-four (72.3%) patients in group I chose to terminate their pregnancies, and uncomplicated medical abor-

Table 1. Comparison of demographics and CA19.9 levels of the groups

	Group I (n: 33)	Group II (n: 33)	Group III (n: 34)	p	p		
					I–II	I–III	II–III
Age (years)	27.2 ± 6.1	26.4 ± 4.8	25.9 ± 5.1	0.583	0.811	0.557	0.911
BMI [kg/m^2]	27.1 ± 4.8	26.9 ± 3.6	24.8 ± 5.8	0.080	0.969	0.100	0.163
Gravida	2 (1–6)	2 (1–4)	2 (0–4)	0.313	0.935	0.184	0.205
Parity	1 (0–2)	1 (0–2)	2 (0–4)	**0.005**	0.634	**0.003**	**0.016**
Live children	0 (0–2)	1 (0–3)	2 (0–4)	**0.004**	0.410	**0.002**	**0.010**
Abortion	0 (0–4)	0 (0–2)	0 (0–2)	0.246	0.099	0.635	0.205
Smoker *n (%)*	3 (9.1)	3 (9.1)	8 (23.5)	0.146	1.000	0.113	0.113
Fetal gender *n (%)* Male Female	 14 (42.4) 19 (57.6)	 17 (51.5) 16 (48.5)			0.450		
GW (weeks)	19.9 ± 2.2	20.5 ± 1.5			0.163		
CA19.9 [U/mL]	17.2 ± 17.0	7.1 ± 5.9	4.7 ± 3.6	**< 0.001**	**0.008**	**0.001**	0.109
WBC count	9.9 ± 2.3	9.7 ± 2.0	7.5 ± 1.6	**< 0.001**	0.901	**< 0.001**	**< 0.001**
Neutrophile	7.3 ± 1.8	7.2 ± 1.8	4.6 ± 1.3	**< 0.001**	0.959	**< 0.001**	**< 0.001**
Lymphocyte	1.8 ± 0.5	1.9 ± 0.4	2.0 ± 0.5	0.135	0.536	0.112	0.612
NLR	4.3 ± 1.2	4.0 ± 1.5	2.4 ± 0.7	**< 0.001**	0.691	**< 0.001**	**< 0.001**

BMI — body mass index; AFP — alpha-feto protein; MoM — multiple of the median; WBC — white blood cell; NLR — neutrophile-to-lymphocyte ratio; GW — gestational week. Values are given as mean ± standard deviation and median (range). A *p* value < 0.05 is considered as statistically significant

tions using misoprostol were performed on all of them. Two of the remaining nine (27.3%) patients delivered vaginally, while seven patients gave birth via cesarean section. Birth weights and 5-minute Apgar scores in group I ranged from 2590–3890 g and 7–10 points, respectively. In group II, there were more vaginal deliveries than cesarean births (24 *vs.* 9). Birth weights and 5-minute Apgar scores ranged from 1650–3980 g and 0–10, respectively, in this group. There were also three patients with fetal growth restriction, one patient with stillbirth, and one patient with a preterm birth in group II. A history of previous cesarean section and fetal distress were the most common indications for cesarean section in both groups.

The mean maternal serum CA 19-9 levels were 17.2 ± 17.0, 7.1 ± 5.9, and 4.7 ± 3.6 in groups I, II, and III, respectively ($p < 0.001$) (Fig. 1). When a subgroup analysis was performed, a weak but significant difference was observed between the meningocele and myelomeningocele cases in terms of mean CA 19-9 levels (10.2 ± 11.9 *vs.* 20.2 ± 18.3, respectively; $p = 0.046$). ROC curve analyses demonstrated that the AUC indicative of the CA 19-9 value for discriminating NTDs in pregnant patients was 0.762, with a confidence interval (CI) of 0.650–0.874 (Fig. 2). This difference was statistically significant ($p < 0.001$). According to the highest Youden index, the cut-off values for CA 19-9 were 9.6 IU/mL at 70% (51–84%, 95% CI) sensitivity and 84% (74–92%, 95% CI) specificity (Tab. 2). There were no correlations between CA 19-9 and other numerical variables, except for a significant correlation between CA 19-9 and NLR in group I (correlation coefficient: 0.335, $p = 0.001$). No significant correlation was observed between CA 19-9 and NLR in the other groups.

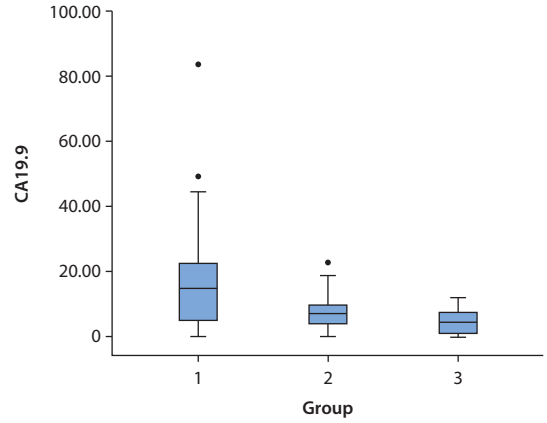

Figure 1. Distribution of CA19.9 values between the groups

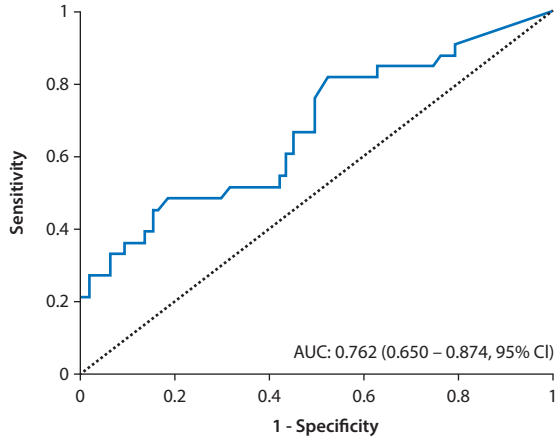

Figure 2. ROC curve analysis of CA 19-9 for the diagnosis of spinal NTDs

DISCUSSION

The present study aimed to evaluate the diagnostic value of maternal serum CA 19-9 levels in pregnancies complicated by spinal NTDs. We found that CA 19-9 was significantly increased in pregnancies with NTDs. According to this study, the CA 19-9 may be used as a diagnostic marker in these pregnancies.

Various physiological and pathological conditions, such as renal failure, pancreatic and hepatobiliary disease, lung disease, systemic disease, inflammation, menstruation, and pregnancy, can induce significant variations in plasma concentrations of tumor markers [9, 10, 18]. These markers have previously been investigated in the biologic fluids of healthy pregnant and non-pregnant women. The presence of considerable concentrations of tumor markers during pregnancy may be attributed to their involvement in biological functions associated with fetal development, differentiation, and maturation [19]. One of these tumor markers, CA 19-9, is mostly used as a serum marker for malignancies, but it is also present in the serum and amniotic fluid of pregnant women [20]. In a prospective longitudinal study, serum concentrations of CA 125, CA 15-3, CA 19-9, and CEA were followed in healthy pregnant women throughout pregnancy [21]. No significant difference was observed in serum maternal serum CA 19-9 levels between the first and second trimesters; however, third trimester CA 19-9 levels showed

Table 2. Sensitivity and specificity of CA19.9 for discriminating meningomyelocele

	AUC (95% CI)	Cut-off value	Sensitivity (95% CI)	Specificity (95% CI)	p
Ca19.9	0.762 (0.650–0.874)	9.6	70 (51–84)	84 (74–92)	< 0.001

AUC — area under the curve; CI — confidence interval. A *p* value < 0.05 is considered as statistically significant

a significant increment compared to the first trimester. On the other hand, Thouitou et al. [18] reported no significant elevations in serum CA 19-9 values throughout pregnancy.

Tayyar and Tutus [22] reported that maternal serum and amniotic fluid CA 19-9 levels were elevated in primigravidas and in pregnant women with female fetuses. In that study, genetic amniocentesis was performed during weeks 16–20 of the pregnancy. Similarly, we carried out the present study at around 20 weeks of pregnancy. There were no significant differences between the pregnant groups in terms of maternal age and fetal gender. Multiparity, which may affect serum levels of CA 19-9, was significantly higher in the validation cohort. However, the pregnant groups were similar regarding the median parity in our study.

It has been shown that trophoblast cells express CA 19-9, and amnion cells produce and secrete it into the amniotic cavity throughout gestation [23, 24]. There have been several reports that CA 19-9 might be used as a diagnostic marker in some pathological pregnancies. Amhad et al. [25] found that low AFP and increased CA 19-9 can be used as tumor markers in the diagnosis of molar pregnancies in conjunction with clinical and sonographic findings and serum levels of human chorionic gonadotropin. It has also been suggested as a diagnostic marker for Down syndrome. However, there are conflicting reports on the use of CA 19-9 as a potential screening marker for the prenatal diagnosis of Down syndrome [26, 27]. To the best of our knowledge, the present study on the relationship between CA 19-9 and NTDs is the first such report in the literature. A previous study showed that exposure of the spinal cord to amniotic fluid leads to inflammation and tissue injury, although these histological findings are greater in the presence of meconium [28]. In this context, we propose that the exposure of spinal cord defects to the amniotic fluid may initiate local inflammation, resulting in an elevated CA 19-9 level. Inflammatory cell infiltration was shown in one of the patients who accepted a histopathological examination in our study (Fig. 3). Similar to the AFP mechanism, the oncofetal antigen CA 19-9 may also diffuse to the maternal circulation through the amniotic fluid from open NTDs.

This was a preliminary study evaluating the diagnostic value of CA 19-9 in pregnancies complicated by spinal NTDs. The present study demonstrated that NLR had a significant positive correlation with maternal serum CA 19-9 levels, which suggests an inflammatory process in the pathophysiology of the disease. The main limitation of this study was the small number of patients included. We also did not know the baseline CA 19-9 levels of the patients. However, we think that the addition of a validation group, which consisted of non-pregnant women, reduced this requirement. Additionally, ultrasonography is already a validated diagnostic tool for prenatal diagnosis of NTDs, and AFP is a known diagnostic serum marker. However, it is sometimes difficult to diagnose these types of anomalies, especially for inexperienced obstetricians, on fetal neurosonography. AFP values are sometimes found to be normal in small and closed defects or elevated in some other fetal anomalies.

We think that CA 19-9 may be a promising noninvasive marker for NTDs. It is a simple and easily applicable marker that can be used in combination with other biochemical markers, such as AFP, to improve the diagnostic accuracy of prenatal screening tests for this type of congenital anomaly. This insight can lead to further studies and further hypotheses to test the diagnostic potential of CA 19-9 in NTDs.

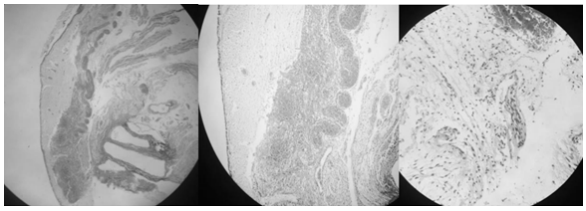

Figure 3. Submeningeal hemorrhage and inflammatory cell infiltration are shown (H.E. 10x–40x)

CONCLUSIONS

To conclude, the basic screening for fetuses with NTDs is ultrasound with a high detection rate. Biochemical markers may only serve as supplementary factors in the diagnosis, especially in the era of high resolution ultrasound machines and fetal magnetic resonance imaging.

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Conflict of interest

The authors declare no conflict of interest.

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