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The Role of Matrix Metalloproteinase As Biomarkers For Neural Tube Defect

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ABSTRACT

Neural Tube Defect (NTD) is one of the most common congenital malformations. It is crucial to determine the prognostic, predictive, or therapeutic genetic factors for preventing NTD. The formation of the extracellular matrix (ECM) plays an essential role in migrating neural crest cells. Matrix metalloproteinases (MMPs) play a significant role in cell migration in ECM organization. The role of expressions and activation of MMP in NTD is unknown. This study aimed to investigate the roles of MMP-1, -2, and 9 gene expressions as biomarkers for NTD.

Peripheral blood samples and NTD tissues were collected from 40 newborn babies diagnosed with NTD, which were also divided into subgroups based on pathology, and peripheral blood samples from only 20 healthy babies were taken for control. After total RNA isolation from blood and tissues, MMP-1, -2, -9 gene expressions were analyzed by Quantitative Real-Time PCR (RT-PCR).

There was no difference between the control group and the NTD group in terms of MMP expressions in blood samples ($p>0.05$). A statistically significantly higher MMP-1 expression was found in Meningocele and Myeloschisis than in Encephalocele ($p=0.014$). A significant difference was found between the tissue and blood samples of the Meningomyelocele patient group regarding MMP-9 expression ($p=0.019$). There was no significant relationship between Ca2+, B12, and Folate levels, NTD, and MMP genes expressions ($p>0.05$).

Even though MMP genes were not different between control and NTD groups, they were found to vary between different subgroups and can serve as biomarkers.

Keywords: Neural Tube Defect, MMP-1, MMP-2, MMP-9

Introduction

Congenital defects are pathological conditions whose causes cannot be determined precisely but directly affect societies' development and sociological structure (1). It is reported that 3% of newborns have congenital anomalies, corresponding to 295,000 new births resulting in death, more than this rate results in long-term disability in the World (2).

One of the most common and severe clinical congenital defects is Neural Tube Defect (NTD). NTD is a congenital malformation related to the central nervous system caused by the failure of the neural tube to close by the 3rd and 4th weeks of intrauterine life (3). NTD is a complex disease in which both genetic and environmental factors play a role in its pathophysiology and may result in death in the prenatal and postnatal periods (4). The incidence of NTD worldwide varies between societies (5). The NTD prevalence in Turkey is shown to be higher than in Europe and the USA (6,7).

The reasons for the formation and development of NTD and the underlying biological mechanisms have not been fully defined. However, in general, it is reported that diabetes mellitus, hormonal imbalances, teratogens, folic acid deficiency, some drugs (such as analgesics, and antiepileptics), excessive use of vitamin A, hypothermia, hyperthermia and, other environmental factors may be effective among the causes of NTD development (8, 9, 10, 11, 12).

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Among the causes that may have a role in NTD development, MicroRNAs, genes involved in the folic acid pathway, and glycaemic dysregulation, certain drugs, and thermal dysregulation can be counted (13). MMPs, which are endopeptidases dependent on Zn^{2+} and Ca^{2+} ions, are the primary matrix proteases in the extracellular matrix (ECM) (14). ECM provides structural support to tissues and cells. ECM synthesis, degradation, and remodeling processes regulate cell migration, differentiation, proliferation, and tissue morphogenesis. The structural contents of the ECM directly affect the cell-cell and cell-ECM interaction within the tissue (15). MMPs play an important role in these biological processes of the ECM. Cell migration and ECM structuring also play an important role in the pathophysiology of NTD (16). So, MMPs may have important roles in the development of pathological tissues such as NTD, in which the processes of cell migration, differentiation, cell-cell interaction, and tissue integrity are involved. Because the presence and activity of MMPs directly affect the cell-ECM relationship. The activity of MMPs plays a primary role in the invasion and metastasis of cancer cells. Therefore, MMPs have the potential to be biomarkers in the diagnosis and treatment of diseases due to their important roles in tissue integrity. (17). The finding out of this potential may contribute to the diagnosis of diseases involving the tissue integrity of MMPs such as NTD.

To our best knowledge, there are no studies in the literature regarding the role of MMPs in tissue remodeling in NTD. Our study, it was aimed to investigate the possible roles of MMP-1, MMP-2, and MMP-9 gene expressions, which are reported to have important roles in cell migration, and so can affect NTD formation. This study showed for the first time that MMPs may have an important role in the pathophysiology of NTD and that MMP expressions may differ between disease subgroups.

Material and Methods

Ethical statement: This study was conducted according to the ethical principles of medical research involving human subjects according to the Declaration of Helsinki. Our study was approved by the Local Ethics Committee of Yüzüncü Yıl University Faculty of Medicine with the number TTU-2018-694. Ethical consent was obtained from the heirs of all the babies.

Collecting Blood and Tissue Samples of Patient: Peripheral blood samples (2 ml) from 40 patients with a diagnosis of NTD (these were also divided into subgroups based on pathology) and 20 healthy babies were obtained to analyze the difference in gene expression between patients with NTD and normal healthy individuals' blood. To analyze the likely differences between MMP gene expression in blood and tissue of each patient, a tissue sample (3-5 mm^3) from the non-skin-covered membrane of the sac (Figure 1) was taken from 40 infants during NTD surgery. B12, Folate and Ca2+ values of the patients, essential clinical parameters in NTD, were biochemically measured.

Total RNA isolation and cDNA synthesis: Total RNA was isolated from the blood and tissue samples taken within the scope of the study by the trizol method. NTD tissues were frozen at -80°C until the number of tissue samples was 10, but RNA was isolated from peripheral blood samples immediately. NTD tissues were cut after helping with a scalpel on ice and RNA isolation was made. The concentration and quality of extracted RNA were examined by a spectrophotometer and 1% agarose gel electrophoresis, respectively. Complementary DNA (cDNA) synthesis was performed using the GeneAll HyperScriptFirst Strand Synthesis Kit (Catalog: 601-005).

Gene Expression Analysis by quantitative Real-Time PCR (qPCR): Expressions of MMP-1,-2 and -9 genes were investigated by the quantitative RT-PCR method. RT-PCR was performed using Biotium Fast-Plus Eva Green Master Kit. The used PCR primers for analyzed genes, F-5'-CGCACAAATCCCTTCTACCC-3', R-5'-GAACAGCCCAGTACTTATTCCCT-3'for MMP-1 gene, F-5'-GCAAGTTTCCATTCCGCTTCC-3', R-5'-CACCTTCTGAGTTCCCACCA- 3' for MMP-2 gene; F-5'-5TACCACCTCGAACTTTGACAG-3' CAGTGAAGCGGTACATAGGG-3' for MMP-9 gene, F-5-GATGGTGGGCATGGGTCAGAAGGA-3' F-5'-CATTGTAGAAGGTGTGGTGCCAGAT-3' for Beta-actin gene that used a housekeeping control gene to normalize the target transcripts. Cycle threshold (Ct) values of complementary DNAs (cDNA) obtained from peripheral blood samples of NTD patients were compared with mean Ct values of healthy control individuals; Ct values of cDNAs obtained from NTD tissues of NTD patients were compared with the Ct values of their own peripheral blood. Relative

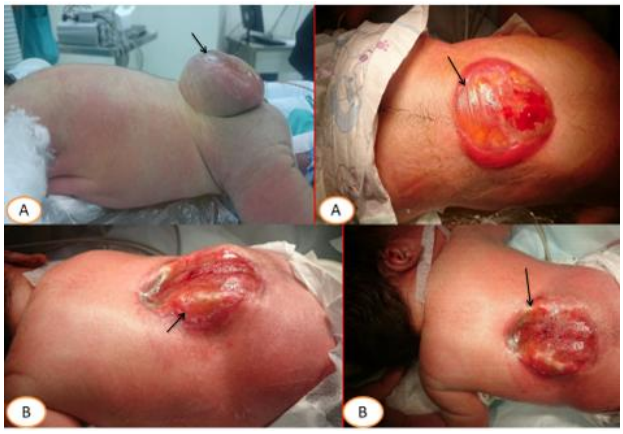

Fig. 1. Showing the location of the tissues taken for study during surgery from babies with spina bifida in the pictures, A) Lumbar Meningomyelocele B) Lumbar Myeloschisis

quantification was performed using the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis: SPSS 20.0 package program was used for the statistical evaluation of the data of the study, and one-way analysis of variance and/or Kruskal Wallis analysis, which is its non-parametric equivalent, was applied to determine the differences between the expression rates obtained from tissue and blood samples between the groups and the control group. Same methods will be used to perform comparative analysis between subgroups of patient population. Multiple comparison tests were used to determine the groups that made the difference. Student's t-test and/or Mann-Whitney U test were used to compare the demographic data of the study in terms of groups, and chi-square or Fisher's exact test was used to examine the distribution of categorical variables. In addition, mean±standard deviation, median (min.-max.), and frequency distributions were used to summarize the results of the study. A *p*-value below 0.05 was considered to be statistically significant.

Results

Demographic and Clinical Characterization of Patients: In this study, NTD peripheral blood and tissue samples of 40 infants diagnosed with NTD were collected. In addition, peripheral blood samples were taken from 20 healthy babies as a control group to compare with the expression levels of the patients' blood samples. The patients' general information and clinical findings are given in Table 1 and Table 2.

Of 40 patients 28 patients were diagnosed with Meningomyelocele (MMC) (70%), 4 with Meningocele (M) (10%), 4 with Myeloschisis (MS) (10%), and 4 with Encephalocele (E) (10%). NTD lesions of the patients were primarily located in the lumbar (46.4%) and least in the sacrococcygeal (3.6%) and cervical (2.5%) regions. Some NTD patients were diagnosed with hypothyroidism (37.5%) and syndromic cases (27.5%). Also, malformations were detected in 72.5% of our patients.

It was determined that the patients' B12, Folate, and Ca2+ values were within the general average limits. It was determined that the lesion sizes of the patients diagnosed with NTD, as MMC, M, MS, and E, were at clinically normal levels and within limits suitable for the diagnosis. Clinically normal levels and limits were determined as 3-10 centimeters.

MMP Gene Expressions: In this study, we determined that MMP gene expressions of all individuals are different and there are differences in MMP expressions between NTD blood and tissue sample of the same patients.

There was no significant difference between the blood samples of the patient and control groups in terms of expression levels of MMP-1, 2 and 9 genes ($p=0.160$ for MMP-1; $p=0.674$ for MMP-2 and $p=0.207$ for MMP-9, respectively, Table 3). When the expressions of each of the MS, MMC, M and E subgroups in the blood samples were compared with the control, no difference was found in the expressions of the MMP-1,2 and 9 genes ($p=0.070$ for MMP-1; $p=0.175$ for MMP-2 and $p=0.175$ for MMP-9, respectively, Table 4).

When the expressions of MMP-1, 2 and 9 genes detected in the tissues of MS, MMC, M and E subgroups were compared with each other according to delta Ct (ΔCt) data, a significant difference was found between M and E, and MS and E in terms of MMP-1 gene expression ($p=0.014$; $p=0.014$, respectively). According to this result, higher MMP-1 expression was detected in the M subgroup compared to the E subgroup and in the MS subgroup compared to the E subgroup.

We compared the expression levels in tissues and blood of MS, MMC, M and E subgroups using delta Ct (ΔCt) data and found a significant difference only in MMP-9 expression ($p=0.005$). Accordingly, MMP-9 expression in the tissues of MMC patients was significantly higher than in the blood sample of the same patient group ($p=0.019$).

When the relationship between Ca^{2+}, B12 and folate levels and MMP gene expression was

Table 1: General Information of Patients

General Information \ Diagnosis		MMC % (n)	MS % (n)	M % (n)	E % (n)	NTD % (n)
Diagnosis		70 (28)	10 (4)	10 (4)	10 (4)	100 (40)
Gender	Male	43 (12)	50 (2)	50(2)	75(3)	47,5(19
	Female	57 (18)	50 (2)	50 (2)	25(1)	52,5
	Total	100 (30)	100 (4)	100 (4)	100 (4)	100
Maternal Age±SD		28,92±5,43 (25)	32,75 ±9,5(4)	28 ±2,64(4)	35,5±2,12 (4)	30,61± 6,21
Lesion Location	Lumbar	46,4 (13)	50 (2)			37,5
	Lumbosacral	32,1 (9)	25 (1)	25 (1)		27,5
	Occipital				100 (4)	10 (4)
	SacrococcygealLl	3,6(1)				2,5 (1)
	Cervical		25 (1)			2,5(1)
	Thoracolumbar	17,9 (5)		75 (3)		20 (8)
Additional malformation to NTD	Yes	75(21)	100(4)	0(0)	0(0)	62,5
	No	25(7)	0 (0)	100 (4	100(4)	37,5 (15)
Mean Lesion Size (cm x		5,1X5,17	1,8X2,1	6,25X7	4X4	4,3X4,
*Ca^{2+} (mg/dl) ±SD		9,08±0,62	8,60±0,80	8,63±0,49	7,75±0,35	8,91±0
**B12 (pg/ml) ±SD		236,44±103,4	287,75±207,8	352,00±85,0	289,00±33,9	250,09
***Folate (ng/ml) (±SD		13,45±3,05	10,95±3,41	16,00±3,41	13,00±7,64	13,3±3 ,68

SD: standard deviation *Normal values: (8.4-10.8),** Normal values: (187-583), Normal values (3-17), Meningomyelocele: MMC, Meningocele: M, Encephalocele: E, Myeloschisis: MS, NTD: Neural Tube Defect

analyzed, Pearson correlation coefficients were calculated to examine their relationship with expression levels in tissues, and no statistically significant association was observed. Therefore, the increase or decrease of the Ca^{2+}, B12, and folate levels did not cause a significant change in terms of other variables.

No statistically significant correlation was found between gender and Ca^{2+}, B12, Folate and MMP Gene expression. Accordingly, it was determined that being male or female did not affect these variables (*p*-values range from 0.177 to 0.784).

When the presence and absence of malformations and Ca^{2+}, B12, folate levels, and MMP gene expressions were compared, there was no difference between the presence and absence of malformation in terms of variables (p values from Chi-square and/or Fisher exact tests ranged from 0.349 to 0.593).

Discussion

Genetic and environmental factors play an important role in the formation and development of NTD, which exhibits a multifactorial inheritance. Due to the heterogeneous nature of NTD, its pathophysiology has not been fully elucidated in terms of molecular and physiological aspects (18). However, considering the medical and social problems caused by NTD, there is a need to elucidate NTD molecular structure in terms of diagnosis and therapy. However, considering the medical and social issues caused by NTD, there is a need to enlighten the predictive, prospective, and/or therapeutic molecular biomarkers for NTD treatment and prevention. For this, the molecular mechanism of NTD needs to be clarified. This way, we can develop new therapeutic medical treatments.

Diabetes mellitus (19), hormonal imbalances, teratogens, folic acid deficiency (20), some drugs (21), and excessive use of vitamin A can be counted among the environmental and medical reasons that may lead to the formation and development of NTD (22). It is stated that microRNAs (23, 24), folic acid pathway genes and PCP (Planary Cell Polarity) genes may be among

Table 2: Clinical Findings of NTD Patients

PATIENT NO	AGE *	MOTHER AGE	Gender**	DIAGNOSIS	LESION PLACEMENT	LESION SIZE (cm)	ADDITIONAL DISEASE	Ca^{2+}	B12	FOLAT	MALFORMATION
P1	1M	30	M	MMC	LUMBOSACRAL	6X8	NO	9,7	167	12,5	NO
P2	N	27	F	MMC	THORACOLOMBER	7X8	NO	9,3	309	5,9	NO
P3	N	27	M	MMC	LUMBAR	6X4	SYNDROMIC, HYPOTHROIDIA	8,8	265	14,9	YES
P4	N	25	F	MS	THORACOLOMBER	5X6	HYPOTHROIDIA	8,7			YES
P5	N	26	F	MMC	LUMBOSACRAL	5X5	NO	8,90	104	14.40	YES
P6	N	37	M	MMC	THORACOLOMBER	6X7	SYNDROMIC	9,3	131	10,6	YES
P7	N	41	M	MMC	LUMBAR	5X4	NO	9	263	15,90	YES
P8	N	28	F	MMC	LUMBAR	6X4	HYPOTHROIDIA	8,2	192	12,7	YES
P9	1Y	42	M	MMC	LUMBAR	3X2	NO	10,1	83	15	YES
P10	N	24	M	MMC	LUMBOSACRAL	5X4	NO	8,6	232	11	NO
P11	N	34	M	E	OCIPITAL (CRANIAL)	4X3	HYPOTHROIDIA	8,7	187	11,3	YES
P12	N	35	M	MMC	LUMBAR	4X2	HYPOTHROIDIA	9,5	131	16,2	YES
P13	N	25	F	MS	THORACOLOMBER	9X4	HYPOTHROIDIA, SYNDROMIC	8,4	354	19,8	YES
P14	N	34	M	MMC	LUMBAR	5X5	NO	7,5	136	15,7	YES
P15	N	30	M	MS	THORACOLOMBER	6X12	HYPOTHROIDIA	8,3	266	15	NO
P16	N	29	F	MMC	LUMBOSACRAL	8X7	HYPOTHROIDIA	8,6	240	12,6	YES
P17	1M	29	F	MMC	THORACOLOMBER	4X6	NO	10,1	229	11,8	NO
P18	N	40	F	M	LUMBAR	2X3	NO	7,8	230	13	YES
P19	N	23	F	MMC	LUMBOSACRAL	4X5	SYNDROMIC	9,4	137	15,2	YES
P20	N	38	F	MMC	LUMBAR	7X8	HYPOTHROIDIA, CARDIAC PATHOLOGY (ASD))	8,5	333	11,8	YES
P21	N	30	F	MMC	LUMBAR	4X5	NO	9,5	347	16,6	YES
P22	N	25	F	MMC	THORACOLOMBER	6X5	HYPOTHROIDIA	10,2	230	13,7	YES
P23	N	23	F	MMC	LUMBAR	3X4	NO	9,6	370	14,6	YES
P24	N	24	M	MMC	LUMBAR	7X6	HYPOTHROIDIA	9,1	144	17,5	NO
P25	N	37	M	E	OCCIPITAL	8X8	HYPOTHROIDIA	7,5	313	7,6	YES
P26	N	40	F	MMC	LUMBOSACRAL	4X5	NO	8,9	255	10	YES
P27	N	23	M	MMC	LUMBAR	4.5X3	NO	9,1	350	13,2	YES
P28	N	40	M	M	LUMBAR	2X2	HYPOTHROIDIA	8,50	594	13	YES
P29	N	27	M	MMC	THORACOLOMBER	5X6	SYNDROMIC	8,3	542	16,1	YES
P30	N	29	F	E	OCCIPITAL	2X2	SYNDROMIC, TWINS	8	272	15,8	YES
P31	N	25	F	MMC	LUMBAR	4X5	CARDIAC PROBLEM	9,5	285	11,9	YES
P32	N	30	F	MMC	LUMBAR	3X4	NO	9,7	250	15,6	YES
P33	N	31	F	M	CERVICAL	2X2	NO	8,4	135	15,4	YES
P34	N	29	F	MS	LUMBOSACRAL	5X6	SYNDROMIC	9,2	436	13,20	YES
P35	N	25	F	MMC	SACROCOXYGEAL	7X6	SYNDROMIC	9,5	280	11,3	YES
P36	N	39	F	MMC	LUMBOSACRAL	6X4	SYNDROMIC	8,6	173	18,3	NO
P37	N	34	M	E	OCIPITAL(CRANIAL)	2X3	SYNDROMIC	8	265	18,4	NO
P38	N	22	M	MMC	LUMBOSACRAL	4X5	NO	8,3	98	17,2	YES
P39	N	31	F	MMC	LUMBOSACRAL	6X8	SYNDROMIC (JLS)	8,6	173	8,7	NO
P40	N	20	M	M	LUMBOSACRAL	1.5X1.5	NO	9,7	192	2,9	YES

Abbreviations: Newborn: N, *Year: Y, Monthly: M, Gender: G, Meningomyelocele: MMC, Meningocele: M, Encephalocele: E, Myeloschisis: MS, **M: Male, F: Female, JLS:Jarcho-Levin Syndrom

Table 3: MMP genes Expression between control and NTD patients in bloods sample

Sample	Genes	Control	Patients (All NTD)	*p value*
Blood	MMP-1	1(1-1)	0.23(0-50.62)	0.160
	MMP-2	1(1-1)	1.23(0.01-59.72)	0.674
	MMP-9	1(1-1)	1.28(0-13.02)	0.207

NTD:Neural Tube Defect

Table 4: MMP genes Expression between NTD subgroups in bloods sample

Sample	Genes	Control	MS	MMS	M	E	*p value*
Blood	MMP-1	1(1-1)	0.15(0.03-0.21)	0.29(0-50.62)	0.18(0.08-1.95)	0.19(0.1-1.05)	0.070
	MMP-2	1(1-1)	1.06(0.7-19.6)	1.34(0.01-59.72)	0.63(0.28-1.77)	1.43(0.21-3.75)	0.175
	MMP-9	1(1-1)	1.28(1-2.33)	1.52(0-11.78)	1.29(0.74-13.02)	0.57(0.15-1.83)	0.175

MS: Myeloschisis, MMS: Meningomyelocele, M: Meningocele: E: Encephalocele

the molecular genetic factors that may cause NTD formation (19).

The structure, formation and development of the ECM play an important role in the pathophysiology of NTD (18). It is very difficult to define the role of ECM structure in NTD. Most of the proteins in the ECM activate several important cellular signaling pathways related to cell polarity, cell migration, cell adhesion, and cytoskeleton that play a role in NTD formation (18). MMPs play a role in cell migration, metastasis (25), vascularization, and wound healing (26) by causing the degradation of various proteins in the ECM. Because NTD results from the inability of the neural plate to fold and close, The investigation of MMPs, which have been reported to play a role in ECM formation (27), may provide crucial clues to explain the NTD pathogenesis and prevent NTD formation. Therefore, it is aimed to determine the possible roles of MMP-1, 2 and 9 gene expressions in NTD in this study. The main reason for choosing MMP-1, 2, and 9 genes in this study are that these genes have important roles in cell migration and wound healing (28, 29). Copp et al. (19) classified the genetic factors that may be at the basement of multifactorial pathology of NTD as a) genes involved in the folic acid pathway and b) genes involved in the PCP pathway. There is no compatibility between the studies related to the use of folate and genetic polymorphisms (30, 31, 32). The view that folate deficiency and the dose used alone majorly affect the development of NTD is controversial, and it is stated that Folate deficiency is a risk factor for NTDs, but does not cause NTD by itself (19). In our study, no relationship was found between the amount of folate and NTD. In addition, no difference was found in terms of the amount of B12. This result may also be due to the number of patients. Also, it is not sufficient to investigate environmental factors alone to explain the pathogenesis of diseases with multifactorial inheritance. Therefore, investigating more genetic and epigenetic factors may be a more proper approach to identifying biomarkers in diagnosing and treating NTD.

There is no data in the literature regarding the role of MMPs in the development of NTD in humans. Therefore, we can not compare our data with other literature. MMPs may play a role in cell migration and orientation through the effect of ECM organization. Thus, cells can be released after ECM degradation as in cancer cells. However, MMP overexpression in the embryonic period may have disrupted cell migration and orientation and blocked tissue development by inhibiting the interactions of cells with each other and the matrix. We investigated MMP expression in the tissue samples that may contain Neural Crest cells (NCC) aside from peripheral blood. NCC cells are primarily responsible for the folding of the neural plate, "the Convergent extension process", and thus the formation of NT. The increase in MMP expression here may have blocked neural folding by the polarity and migration of NCC. In this study, we detected increased MMP-9 expression in NTD tissues of MMC group patients compared to blood samples according to delta ct data. Since the number of MMC patients (28 MMC/40 NTD) was higher in the NTD patient group than in the other MS, M and E subgroups, a significant increase in MMP-9 may have been detected in the tissues of the MMC group. A significant difference could be detected if the number of patients was higher in other NTD subgroups. MMP-9 has a vital role in NCC cell migration (29,33). Therefore, our result may have important potential for understanding the pathogenesis of NTD.

It was reported that the expression of MMP-16 increased the migration of NC cells of bird cranial embryos, and when it decreased, it inhibited the migration of NC cells and Epithelial-Mesenchymal Transition (EMT) (25). So, it was concluded that the increase in MMP expression triggers the

formation of NTD (25). In the study of Roth et al. (25), MMP-16 expression was investigated in NC cells of bird cranial embryos. In our study, MMP-1, 2 and 9 genes were investigated in human blood and NTD tissues (not only cranial). For this reason, it is possible that the results of Roth et al. (25) and the results obtained in this study differ. The types of organisms used in studies to explain NTD pathogenesis and neurulation are important in terms of the usability of the results. Copp et al. (19) reported that there are more than 250 mouse models to understand the neurulation mechanism and genetically different mutant models may present different features from each other. It is stated that all human cells do not have the same genomic structure and that peripheral blood studies cannot fully reflect the disease under investigation in the study by Gottlieb et al. (34). This difference between tissues has also been confirmed epigenetically (35). Therefore, NTD-specific organoid-like models are needed in studies to explain NTD pathogenesis.

Monsonego-Ornan et al. (33) reported that increased MMP-9 expression plays an important role in the separation and migration of NC cells by degrading the epithelial cell junction protein E-cadherin and the laminin in tissue taken from NC cells in bird embryos (36). The reason why our study result was different from Monsonego-Orman et al.'s study may be due to the fact that the peripheral blood samples in this study were peripheral blood.

This study showed that NTD is not associated with Ca^{2+}, B12, and folate levels. Even though MMP genes were not found to be statistically related to development of NTD, MMP genes were found to vary between different subgroups and can serve as biomarkers. More NTD tissue samples should be studied to investigate the effects of MMP genes and similar genes to reveal the importance of their roles. More detailed studies are needed in organoid-like, tissue culture-based in vitro models that can represent the human NTD structure. Animal models may not be adequately representative of human NTD due to variations in genetic, epigenetic, proteomic, and metabolomic features. This is the first study investigating MMP genes' roles in NTD cases.

Ethics Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Our study was approved by the Local Ethics Committee of Yüzüncü Yıl University Faculty of Medicine with the number TTU-2018-6941

Abbreviations List:

Neural Tube Defect (NTD)
Meningomyelocele (MMC)
Meningocele (M)
Myeloschisis (MS)
Encephalocele (E)
Planary Cell Polarity (PCP)
Extracellular Matrix (ECM)
Metalloproteinases (MMP)
Metalloproteinases-1 (MMP-1)
Metalloproteinases-2 (MMP-2)
Metalloproteinases-9 (MMP-9)
Complementary DNA (cDNA)
Real-Time PCR (qPCR, RT-PCR**)**
Cycle threshold (Ct)
Neural Crest cells (NC cells)
Epithelial-Mesenchymal Transition (EMT)
Membrane Type 1-MMP (MT1-MMP)
Protein-Kinase 7 (PTK-7)

Credit Authorship Contribution Statement:

MEA: Methodology, Laboratory Studies, Investigation, Writingeoriginaldraft,

FNDE: Laboratory Studies, Writing, review & editing

FT: Laboratory Studies

VY: Laboratory Studies

OT: Methodology, Investigation, Evaluating results

MA: Methodology, Investigation, Evaluating results

MT: Laboratory Studies, Writing, review & editing

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Declarations:

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