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Do the trace elements play a role in the etiopathogenesis of developmental dysplasia of hip?

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Abstract. – OBJECTIVE: Alterations in the connective tissue of the hip joint capsule and ligaments might account for the increased laxity seen in patients with developmental dysplasia of the hip. The tensile features of the connective tissue depend on collagen. A number of prior studies have noted the association between the trace elements and collagen biosynthesis. The aim of this research is to determine whether there exists an association between the trace elements and developmental dysplasia of the hip.

PATIENTS AND METHODS: This investigation included 27 patients with developmental dysplasia of the hip (18 females and nine males; mean age 24.3 ± 6.3 months, range 18–36 months) and 26 healthy controls (15 females and 11 males; mean age 23.8 ± 5.4 months, range 18-36 months). The levels of the serum trace elements in the groups were statistically compared.

RESULTS: The Cu levels of the patients with developmental dysplasia of hip were statistically higher than those of the control group ($p<0.05$). The Zn, Fe, Mg, and Mn levels of the patients with developmental dysplasia of hip were statistically lower than those of the control group ($p<0.05$).

CONCLUSIONS: We found an association between developmental dysplasia of the hip and the serum trace element levels. We, therefore, believe that the trace element levels may shed light on the etiopathogenesis of developmental dysplasia of the hip. This work should be supported by future studies concerning the causes of the alterations in the serum trace element levels seen in patients with developmental dysplasia of the hip.

Key Words:
Trace element, Developmental dysplasia of the hip, Collagen turnover.

Introduction

Developmental dysplasia of the hip (DDH) is a musculoskeletal condition seen in newborns. The reported prevalence of DDH upon physical examination at birth ranges from 0.16% to 2.85%, although the prevalence of persistent abnormalities after the first few days of life, as reported in a meta-analysis of studies conducted in American and European populations, is 0.13%[1]. DDH is related to early-onset osteoarthritis of the hip in adults[2,3]. DDH implies dysplasia of the acetabulum (incomplete development) or instability of the hip, such that the femoral head can be partly or totally moved out of the hip joint[4]. Alterations in the connective tissue of the hip joint capsule and ligaments might account for the increased laxity seen in patients with DDH. The tensile features of the connective tissue depend on collagen[5].

The trace elements represent a vital part of various biological structures. Although a number of studies have investigated the relationship between the trace elements and collagen biosynthesis[6-11], to the best of our knowledge, no prior researches in the English literature have investigated the trace elements in relation to collagen metabolism in DDH. In light of the above facts, the serum trace element levels may be an important factor in the pathogenesis of DDH.

Therefore, we evaluated the levels of the serum trace elements in patients with DDH and we compared them to the levels seen in the control subjects. Our aim was to investigate whether there exists an association between the trace elements and DDH.

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Patients and Methods

Study Population

This cross-sectional prospective study was conducted in the Department of Orthopaedics and Traumatology and Chemistry at Yuzuncu Yil University between June 2013 and May 2014.

We included 27 patients with DDH (18 females and nine males; mean age 24.3 ± 6.3 months, range 18-36 months) and 26 healthy controls (15 females and 11 males; mean age 23.8 ± 5.4 months, range 18-36 months) who were free of symptoms. The inclusion criteria were patients aged between 18 and 36 months, isolated DDH, abnormal physical examination findings, and no previous treatment. The exclusion criteria included teratologic dislocation, neuromuscular causes, those having undergone previous treatment, bilateral cases, and patients classified as Tönnis 1. The control subjects were found from among children attending the pediatric outpatient clinic who were totally healthy. The trace element levels of the groups were statistically compared.

Ethical Approval

The study protocol conformed to the principles of the Declaration of Helsinki. Further, the investigation was approved by the local Ethics Committee of Yuzuncu Yil University, and informed consent to participate was obtained from all the participants' families.

Clinical and Radiological Assessment

All physical examinations of all the hips were performed by the same orthopedic surgeon (SG). The Barlow and Ortolani maneuvers were performed in order to determine the hip stability. Further, the Tönnis classification[12] was used to evaluate the radiographic results (Figure 1). All the radiographs of all the patients were evaluated by the same observers (SG and SIG).

Collection of Blood Samples

After fasting for 12 hours overnight, blood samples were taken and then stored on ice at 4°C in the morning. The serum was centrifuged at 3000 rpm for 10 minutes, before being stored at -20°C until the assays were conducted.

Table I. Demographic characteristics of the groups.

	Patients'	Controls'	*p*-value
Age (month)	24.3±6.3	23.8±5.4	>0.05
Affected site (L/R)	20/7	-/-	-
Gender (F/M)	18/9	15/11	>0.05

L: left; R: right; F: female; M: male.

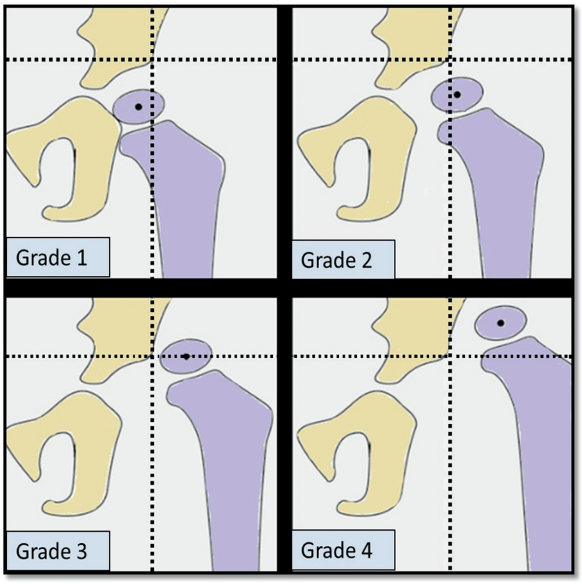

Figure 1. Tönnis classification.

Biochemical Analysis

A H2O2/HNO3 (1:2) mixture (2 ml) was added to the serum samples (0.7 g). The solution was placed in a water bath for 30 min at 70oC and stirred intermittently. Next, more of the H2O2/HNO3 mixture (1 ml) was added, and the solution was transferred into a Teflon vessel bomb, which was then closed. The solution was placed in a microwave oven, and radiation was applied at 450 W for 3 min. Then, more of the acid mixture was added (0.5 mL) and radiation was again applied for 3 minutes. Cooling was applied for 5 minutes. Then, HNO3 was added (2 mL of 0.1 mol/L), and the mixture was transferred into a Pyrex tube. After centrifugation, the trace element levels were determined. The atomic absorption spectrophotometer technique was used to conduct the measurement with a UNICAM-929 spectrophotometer (Unicam Ltd., York Street, Cambridge, UK).

Statistical Analysis

All descriptive statistics for all the variables (characteristics) were presented as the minimum and maximum values, mean, and standard deviation. Student's t-test was used to compare the groups' mean values for the studied variables. To determine the linear relationships among the variables, a Pearson correlation analysis was carried out in each group. The statistical significance

Table II. Trace element levels.

	Groups	N	Mean	Std. Dev.	Std. Error	Min.	Max.	*p*-value
Cu	Control	26	0.753177	0.0791509	0.0155228	0.5777	0.8665	0.05
	Patient	27	2.063178	0.1630540	0.0313798	1.8515	2.4663	
Fe	Control	26	2.099392	0.1068851	0.0209619	2.0111	2.3122	0.05
	Patient	27	1.008422	0.1835097	0.0353165	0.5733	1.2248	
Mg	Control	26	55.62731	2.6676095	0.5231613	50.540	60.540	0.05
	Patient	27	32.35815	3.4572833	0.6653545	25.670	39.630	
Mn	Control	26	0.109715	0.1480573	0.0290364	0.0675	0.8352	0.05
	Patient	27	0.033200	0.0046909	0.0009028	0.0276	0.0422	
Zn	Control	26	2.936385	0.2222111	0.0435792	2.5250	3.3210	0.05
	Patient	27	1.073796	0.1975299	0.0380147	0.6788	1.5325	

Std: Standart; Dev: Deviation.

level was considered to be 5%, and the Statistical Package for the Social Sciences specialized computer program version 13.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical calculations.

Results

The demographic characteristics of both groups are presented in Table I. There were no statistically significant differences between the DDH patients and the controls with regard to either age or gender. The trace element levels of the groups are presented in Table II. The Cu levels of the patients with DDH were statistically higher than those of the control group ($p<0.05$). However, the Zn, Fe, Mg, and Mn levels of the patients with DDH were statistically lower than those of the control group ($p<0.05$).

Discussion

Despite detailed research having previously been conducted, the etiopathogenesis of DDH remains unclear. Abnormalities in the shape of the acetabulum and the femoral head have been found in patients with DDH. Indeed, the acetabulum is too shallow while the femoral head is insufficient in patients with DDH. In addition, joint capsule laxity might be present in DDH patients[13,14]. In fact, the laxity of the joint capsule is the main factor behind DDH. Collagen is a major component of the laxity seen in patients with DDH[15]. The trace elements play a key role in the production of collagen in the human body[6-11]. Further, the trace elements are accepted to be the major constituents of various biological structures.

Copper (Cu) plays a role in the synthesis of collagen as an essential nutrient[5]. The incubation of chicken femurs (embryonic) with 2.5 μM or more of Cu resulted in decreased collagen being seen in the epiphysis and diaphysis, mainly due to the inhibition of collagen production[7]. We found that the serum Cu levels were significantly higher in the patient group than in the control group.

Magnesium (Mg) modulates the synthesis of both collagen and elastin, which are two fibrillar components of the extracellular matrix, as well as the non-fibrillar macromolecules (glycoproteins and proteoglycans). Further, Mg has proven effective in maintaining the structure and mechanical features of elastic fibers, and it is also involved in elastic fiber elastolysis[8]. Thus, we suggest that Mg maintains the structure and function of the hip joint capsule. We found that the serum Mg levels were significantly lower in the patient group than in the control group.

Manganese (Mn) is necessary for numerous enzyme activities. It also plays a role in the synthesis of the proteins involved in connective tissue regeneration. Hence, Mn deficiency can be the reason for bone deformities and defects in chondrogenesis[9]. We showed that the serum Mn levels were significantly lower in the patient group than in the control group.

Zinc (Zn) is one of the most plentiful nutritionally essential trace elements found in the human body. Additionally, a Zn deficiency has been indicated to reduce both collagen and proteoglycan synthesis[10]. We observed that the serum Zn levels were significantly lower in the patient group than in the control group.

Iron (Fe) plays a significant role in collagen maturation, and it is a cofactor for the lysyl and prolyl hydroxylases in collagen synthesis[11]. Our study showed that there was a significant diffe-

rence between the serum Fe levels of the patients with DDH and the control group, since the serum Fe levels were significantly lower in the patient group than in the control group. Therefore, in patients with DDH, these Fe-dependent enzymes might become inactive, and the abnormal metabolism of collagen might occur.

The prolidase enzyme also plays an important role in collagen metabolism. Indeed, the final stage of collagen degradation is mediated by prolidase[16,17]. The prolidase enzyme requires Mn and Zn in order to achieve its maximal activity[18]. Thus, Mn and Zn are considered to be activators of prolidase[19]. Further, prior studies have determined that prolidase enzyme activity may play an important role in collagen biosynthesis[17,20].

Based on the above discussion, we believe that the trace elements may either directly or indirectly affect collagen biosynthesis. Taken together, our results suggest an association between collagen turnover and the trace element levels.

It is important to recognize that the present work involved a relatively small number of patients. Therefore, more studies with larger populations are needed in the future to investigate the serum trace element levels and their relation with collagen metabolism. Moreover, the hip joint capsule trace element level was not measured. Future studies should therefore endeavor to include that measurement.

Conclusions

An association was observed between the serum trace element levels and DDH in the present study. We thus believe that the trace element levels may shed light on the etiopathogenesis of DDH. This study should be supported by further investigations concerning the reasons behind the alterations in the serum trace element levels seen in patients with DDH.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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