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Fibromodulin Level in Association with Clubfoot Disease and Congenital Dislocation of Hip in Children Patients in Najaf Province

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ABSTRACT

Background: The main characterizations of foot deformity, which is called congenital talipes equinovarus, in severity and variation degree depend on complications and congenital malformations. They are classified into four main components such as midfoot cavus, forefoot adducts, heel/hind foot various and hind foot equines.

Objective: This study aimed to estimate some of biochemical markers such as asporin, fibromodulin and tenascin-C in club foot child disease to determine pathological causes of severe deformity of bone, for monitoring complications of bone mineralization and calcification and for early treatment using drugs of choice.

Patients and Methods: 60 Clubfoot patients with dislocation dysplasia of Hip (DDH) disease were included in this study. The samples were collected from Clubfoot Unit and private centers in Holy Najaf, Iraq during the period from December 2020 to February 2021. The patients groups were divided into subgroups according to age, gender, idiopathic, syndrome, Parina score, body mass index (BMI) and clubfoot. The control group included 30 apparent healthy participants and the age was nearly matched with patients' groups.

Results: The level of tenascin-C was increased significantly in clubfoot patients than in control group. Also, tenascin-C level was highly increased in advanced ages (3-5) years in comparison with other ages and in males than in females. Also, high score (6-7) was significantly increased than other scores. The present results showed that club foot with dislocation dysplasia of hip was highly significant compared to clubfoot only and dislocation dysplasia of hip only in all children patients. Moreover, clubfoot syndrome was higher than idiopathic syndrome significantly whereas no differences in tenascin-C level in all subgroups according to BMI (normal, overweight and obese) of children of clubfoot.

Conclusion: Increased levels of tenascin-C is very important as a pathological marker in patients of clubfoot with dysplasia of Hip.

Keyword: Clubfoot, Children, Tenascin-C, BMI, Ages, Gender, Syndrome.

INTRODUCTION

Clubfoot, also known as talipes equinovarus, is one of the most universal human limb disorders, more than 2% of newborns. Also, the deformity manifests as an instep that has an adductus, the midfoot has a cavus, and the posterior foot has an equinovarus [(1)]. Fibromodulin is the primary hormone of fibril formation [(2)]. Fibromodulin and lumican have similar primary components and bind to almost the same site on type I collagen [(3)].

Only fibromodulin and lumican are engaged in tissue-specific management of fibrillogenesis, in addition to tendon. In the vicinity of fibromodulin, lumican was expanded [(4)]. Fibromodulin (FMOD) is one of the extracellular matrix's small leucine-rich proteoglycans (SLRPs). It has many physiological functions including fibrillogenesis, muscle cell formation, cell fate determination, and angiogenesis enhancement [(5)]. Also participates in the pathogenesis of several pathological systemic fibrosis, cancers, and atherosclerotic plaques are examples of such conditions [(6)]. Therefore, this study aimed to estimate some of biochemical markers such as asporin, fibromodulin and tenascin-C in club foot child disease to determine pathological causes of disease by severe deformity of bone and for monitoring complications of bone mineralization and calcification for early treatment using drugs of choice.

PATIENTS AND METHOD

Patients and healthy groups:

The current study included sixty patients suffering from clubfoot and dislocation dysplasia of hip (DDH) disease. The samples were collected from clubfoot unit and private centers in Holy- Najaf /Iraq during the period from December 2020 to May 2021. The patients groups were subdivided into subgroups according to age, gender, idiopathic, syndromic, parina score, body mass index (BMI) and clubfoot. The control group was thirty appear control and the age were nearly matched with patients groups.

Experimental design:

The clubfoot patient's total numbers of sixty clubfoot group was subdivided into seven groups as the following;

1. Ages (1> year; N=36, (1-2year); N=13, (3-5year) N=11.
2. Gender (male; N=40, female; N=20)
3. Pirani score (2-3 degree); N=20, (4-5 degree) N=34, (< 6 degree; N=6)

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4. Clubfoot disease (idiopathic; N=36, syndromic; N =24).
5. Type of clubfoot (clubfoot only; N=20, clubfoot and DDH; N=32, DDH only; N=8).
6. Body mass index (BMI), (normal weight; N=38, over weight; N=13, obese weight; N=10).

Inclusion criteria: Many symptoms in clubfoot the typically present with child the age, body mass index (BMI), syndromic, idiopathic, weight, and the diagnosis was confirmed by blood and full history of each subject was recorded.

Exclusion criteria: No history of clubfoot and neurogenic club foot cerebral palsy and spinal bifida.

Diagnosis:

A baby's clubfoot is often diagnosed during a parent's prenatal ultrasound. As early as 13 weeks during pregnancy, clubfoot can be detected in about 10% of cases. About 80% of clubfoot cases may be identified by 24 weeks, and this percentage keeps rising until delivery. Clubfoot can be noticed and diagnosed as soon as a kid is born if it is not identified before birth. Usually, a physical exam is all that is required to make a diagnosis. Rarely, further tests including x-rays and computed tomography scans may be required (CT or CAT scan) [7, 8].

Collection of samples

5 milliliters of venous blood were collected from patients with clubfoot or congenital dysplasia of the hip and the control group. In the gel tube, 4 ml of blood was allowed to coagulate for 10 minutes at room temperature. After centrifugation, the serum was extracted, sorted, and then transferred into fresh disposable Eppendorf tubes by micropipette and kept at -20O°C. 1 ml of blood was placed in an EDTA tube for complete blood count measurements in a haematology analyzer or CBC [9, 10].

Determination of Pirani score:

The present study depend on Pirani score for assessment of score 0,0.5,or 1 patients according to following; Medial Crease (MC), Curved Lateral Border (CLB), Lateral Head of Talus (LHT), bone marrow aspiration (BMA), posterior crease (PC), empty heel (EH) and rigid equines (PE)[11].

Determination of Body mass index:

The Body mass index of child was calculated by Kids health BMI for child from 1-2 years olds and percentage by weight (pound) and height (inches) [12].

The categories divided into:

1- Healthy (Normal) BMI equal to greater than 5th percentile and less than 85.
2- Over weight from 85 to 95 percentile.
3- Obese: above 95 percentile.

Determination of idiopathic and syndromic clubfoot

The idiopathic clubfoot was clinically characterized by unknown causes and accrue in family of four question at first time and characterized by complex three dimensional of deformity. While, syndromic (secondary) accrue in family and is characterized by constriction of band (known association in which the clubfoot is considered as being rigid, responding poorly to casting, and requiring surgical interventions) and tibial hemimelia [also known as tibial deficiency, which is a condition in which child is born with a tibia (shinbone) that is shorter than normal or missing altogether] (Figures 3-5). Also, diastrophic dwarfism (rare disorder marked by short stature with short extremities) [13].

Determination of development of dysplasia of Hip:

Physical examination during the newborn phase identified DDH. It may be crucial to ask the parents about risk factors. The gold standard for diagnosis is clinical screening, which includes a dynamic hip examination at birth and a follow-up physician assessment at delivery [14]. Asymmetric shortening on the side of the dislocation is a symptom of unilateral dysplasia (Galeazzi sign):

- The afflicted side's affected leg could bend outward. Hip abduction showed tight hip adductors and asymmetrical thigh or gluteal folds.
- The gap between the legs could appear larger than usual.

The use of ultrasonography is advised. Most of the time, an ultrasonography will make the condition clear. Occasionally, an MRI is also utilised. Rarely, CT scans or 3D CT scans are employed.

Estimation of tenascin-C level.

Fibromodulin was estimated using Elisa kits supplied by Elab-scicnce Company (Korea) [15, 16].

Ethical considerations:

The study concept for human studies was approved from Kufa University's College of Science and AL-Saddar Hospital by The Institutional Ethics Committee. Additionally, before taking part in the study, each individual gave written, informed consent.

Statistical analysis

For Windows 2010, SPSS version 23 was utilized to evaluate the study's data. Unpaired sample t-tests was used to compare two groups, and one-way ANOVA tests was performed to compare groups that had been separated based on the parameters that were assessed [17,18]. SPSS version23 have been used to create each and every figure. A statistically significant threshold of significance was set at $P \leq 0.05$ [19, 20].

RESULTS

The results exhibited a significant increase in fibromodulin level (1.5407 ± 0.068029 ng/ml) in patient ($p< 0.05$) compared to control group (0.57123 ± 0.038268 ng/ml) as shown in Figure (1)

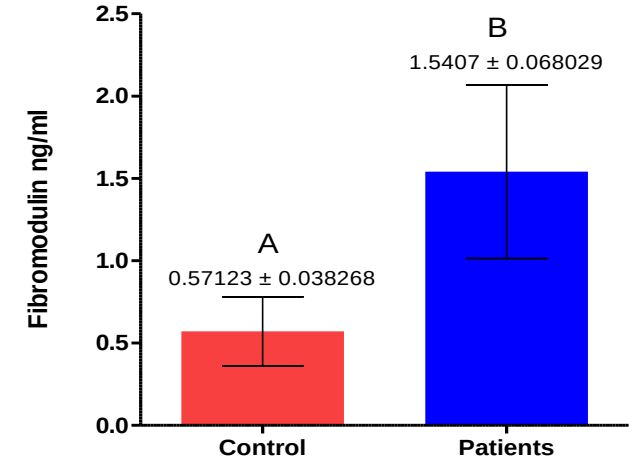

Figure (1): Fibromodulin level in patients and control groups. Different letter refer to significant difference

The results in figure (2) showed significant increase in fibromodulin level in age (3-5) years (2.2503 ± 0.10098 ng/ml) in comparison with age (1-2) years (1.6155 ± 0.052184 ng/ml) and less than (1 years) (1.1137 ± 0.059939 ng/ml).

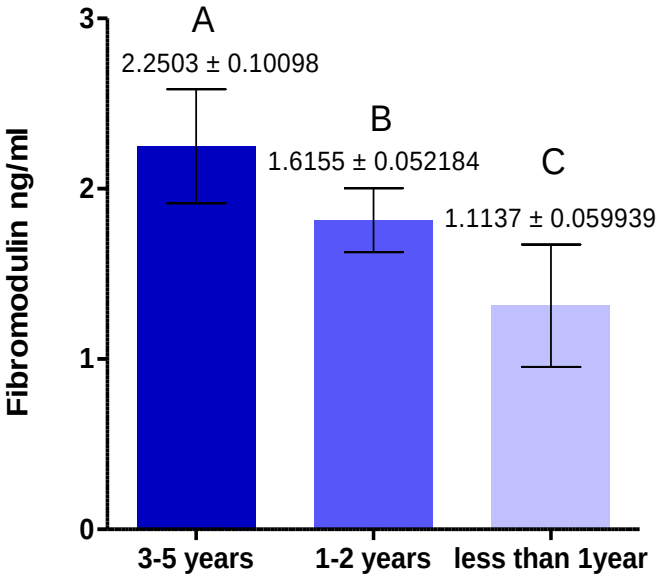

Figure (2): Effect of age on fibromodulin level in patient. Different letter refer to significant difference.

The results in figure (3) exhibited non-significant decrease (P>0.05) in fibromodulin level in female patients group (1.5245 ± 0.074844 ng/ml) compared to male patients group (1.6051 ± 0.086563 ng/ml).

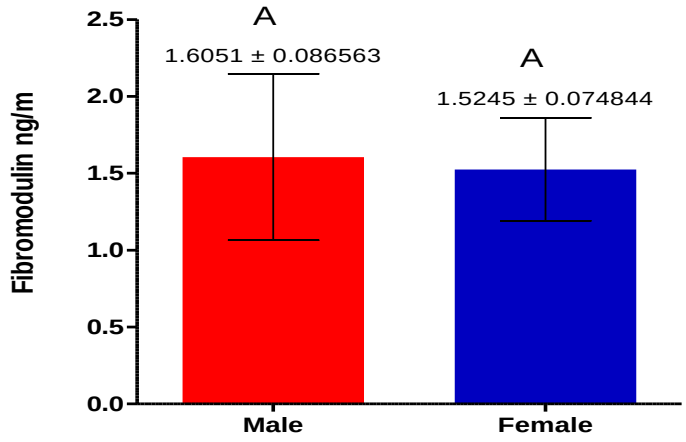

Figure (3): Effect of gender on fibromodulin level in patients. Different letter refer to non-significant difference

The results in figure (4) showed non-significant increase in fibromodulin level in Pirani score (6 score) (1.8363 ± 0.013232 ng/ml) in comparison with Pirani score (4-5 score) (1.7235 ± 0.056921 ng/ml) and significant increase in comparison with Pirani score (1-2-3 score) (1.0690 ± 0.090411ng/ml). The Pirani score 4-5 score also showed significant increase in comparison with Pirani score (1-2-3 score).

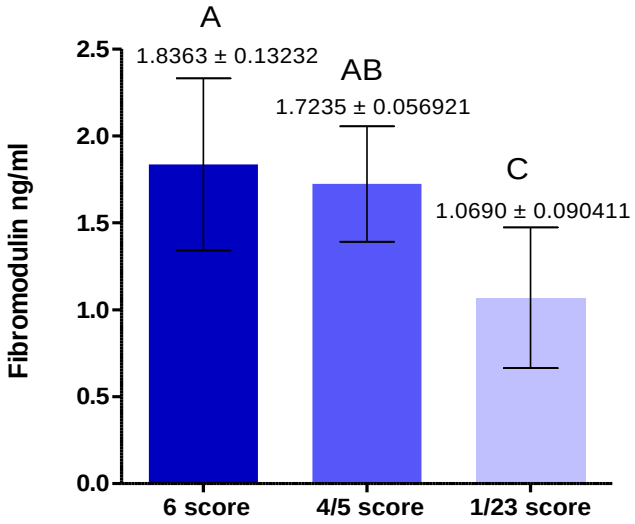

Figure (4): Effect of Pirani score on fibromodulin level in patient. Different letter refer to significant difference and non-significant.

The results in figure (5) referred to significant increase in fibromodulin level in clubfoot and DDH (1.9592 ± 0.070825 ng/ml) in comparison with DDH (1.1585 ± 0.089671 ng/ml) and significant increase in comparison with clubfoot (1.4568 ± 0.11917 ng/ml). The DDH also showed non-significant differences in patient (P>0.05) compared to clubfoot.

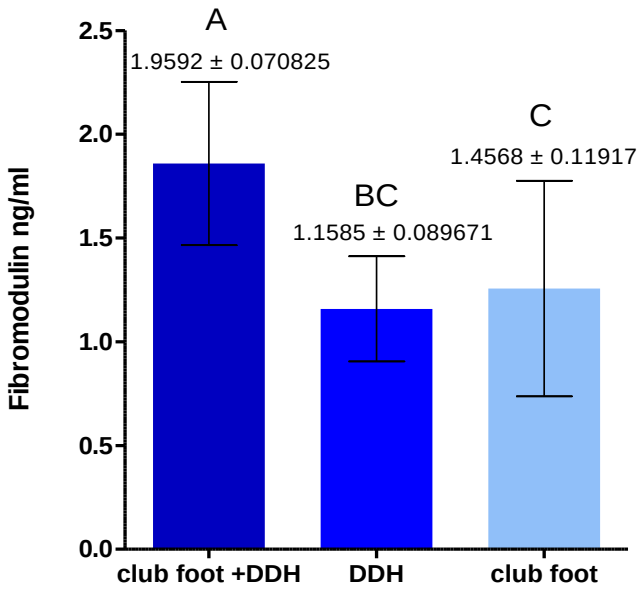

Figure (5): Effect of clubfoot and DDH level in fibromodulin patient. Different letter refer to significant difference and non-significant difference

DISCUSSION

Many studies has been revealed that high level of fibromodulin accelerate a joint disease by propagation of inflammation cascade by activation of complement pathway both classical & alternative and interact with collagen type I and II where FMOD is very necessary for collagen formation [21,22].

Another recent studies has been postulated that severe clubfoot caused increase of fibromodulin by influence on myofibroblast. Cell may lead to deformity and contraction also higher level of fibromodulin lead to fibroproliferative disorder like Dupuytrens contracture and prominent increase of endomysial and perimysial connective tissue [23].

The current study explanation about highly Fibromodulin with age that it may be due to role of proteoglycan constitals who interact with collagen fibril and changes the mechanical properties of tissue especially collagen fiber (abundance and structure) associated with degeneration in advanced age. Former study on fibromodulin in intervertebral disc showed that extracellular matrix (ECM) undergoes degeneration with age and affects the quantity of ECM [24]. The current study agrees with recent research that found no difference in fibromodulin, biglygcan, lumican and versican between the two sex because type I and type III collagen were present as some between males and females and collagenous tissue changes were similar as a properties for mechanical function [25]. Former study on fibromodulin in osteoarthritis has proved that fibromodulin showed high levels in advanced stage than early because it is associated with higher chondrocyte proliferation and stimulating apoptosis [26, 27]. No previous studies about the level in all types of development of abnormal dysplasia of hip with clubfoot complications and severity combined with accumulation of collagen fiber & abnormality of ECM. Therefore, fibromodulin as biomarker level was high due to highly expression in both joints, muscle & hip tissues as a results of these event deformity that was high in bone, cartilage and muscle [28, 29].

CONCLUSION

Increase level of Tinascin-C in clubfoot is very important as a pathological marker with development of dysplasia of Hip patients.

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