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Original article

Role of Musculoskeletal Ultrasound and Matrix Metalloprteinase-2 Serum Level in the Diagnosis of Early Knee Osteoarthritis

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ABSTRACT

Background: Osteoarthritis [OA] is the most common joint disease, causing disability and reduction of quality of life and participation in social activity. Now considered a whole joint disease, OA is characterized by cartilage loss, subchondral bone changes, synovial inflammation and meniscus degeneration.

Aim of the work: To evaluate of the role of musculoskeletal ultrasound and MMP-2 serum in the diagnosis of early knee OA.

Patients and Methods: The present study was conducted on 50 patients with early knee OA who attending the Outpatients Clinic of the Rheumatology Department [Damietta Faculty of Medicine, Al-Azhar University] matched for age and sex with 25 healthy volunteers. Musculoskeletal ultrasound [MUS] was performed by experienced radiologist according to the EULAR recommendations by using a 12–5-MHz linear transducer and Metallo-prteinase-2 [MMp2] was detected by Elisa technique.

Results: MMP-2 is highly significant and overexpressed in patient group and its early detection is positively correlated with weight and body mass index [BMI]. Our results appear that family history, BMI and weight are the main risk factors for the onset of knee osteoarthritis [KOA] in the patient group. MUS clarifies most patients [88% and 80%] have medium osteophyte on left and right respectively, followed by 44% of participants have mild osteophyte on right.

Conclusion: MMP-2 could be considered as a diagnostic biomarker at the early stage of OA. MSU is an excellent imaging technique to detect early osteoarthritis.

Keywords: Metalloproteinase 2; Expression; Musculoskeletal ultrasound; Knee pain; Early Osteoarthritis.

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* Main subject and any subcategories have been classified according to researchers' main field of study.

INTRODUCTION

Osteoarthritis [OA] is the most widely wide-spread joint disease, resulting in reduction of high-quality of life and social activity. OA pathology includes cartilage loss, subchondral bone changes, synovitis and meniscal degeneration[1]. The specific cause of OA is unknown but there are some comorbidities such aging, overweight, obesity, and mechanical stress[2].

OA diagnosis is based totally on signs and symptoms [pain and stiffness], limitation of functional, radiographic findings, and risk elements [age, gender, body mass index [BMI], occupation, family records of OA, records of knee injury, etc] [3].

Knee osteoarthritis [KOA] is frequently accompanied with low grade synovitis, which correlated with joint ache and dysfunction and, which is the most important risk component for the faster development of structural joint deterioration[4].

Musculoskeletal ultrasound [MUS] is utilized as one of the first line modalities for detection of morphological changes in KOA[5].

Since, structural alterations visible late on radiographs, so plain radiography is becoming less frequently utilized in clinical research as a tool for diagnosis of early KOA[6].

US can reveal and assess the minimal structural, abnormalities, which involve the pathophysiology and development of OA, such as articular cartilage, synovial tissue, bony cortex and different soft tissues. It can be used for diagnosing soft-tissue lesions, for grading the severity of OA, and for guiding and monitoring therapy[7].

Biomarkers are quantifiable substances, and their presence can be suggestive of a certain phenomenon or disease [8]. The matrix metallo-proteinases [MMPs] are large family of proteolytic enzymes, consists of at least 28 members[9]. matrix metalloproteinase -2 [gelatinase A, seventy-two kDa type-IV collagenase] which was cultured from human melanoma cells [10]. MMP-2 is commonly expressed in fibroblasts, endothelial and epithelial cells[11]. It is secreted as proenzyme and activated at the cell surface; by the membrane-type metalloproteinase-1 [MT-MMP-1] [12]. Also, the MT-MMP-1, which activates the MMP-2, was extraordinarily expressed in the chondrocytes during OA[13]. In addition, the distribution of MMP-2 and MMP-9 both in normal and osteoarthritic cartilage and in cultured articular chondrocytes is highly expressed[14]. The assessment of MMP-2 can be more appropriate in the diagnosis of early KOA than imaging modalities[15].

AIM OF THE WORK

The aim of this study was to evaluate the role of musculoskeletal ultrasound and MMP-2 serum levels in the diagnosis of early KOA.

PATIENTS AND METHODS

This study included 50 patients with early KOA who attended the outpatient's clinic of the Rheumatology Department [Damietta Faculty of Medicine, Al-Azhar University] and 25 apparently healthy, age and sex matched controls. Informed verbal and written consents were obtained from each participant sharing in the study and the study protocol was approved by Institutional Research Board [IRB] of Damietta Faculty of Medicine, Al-Azhar University, Egypt.

Inclusion criteria: The current work recruited patients with knee pain and early degenerative changes detected by ordinary radiographs grade [0-2], and both genders are included. **Exclusion criteria** were: patients over 45 years of age, patients with history of trauma, pregnant females, patients with any knee pathology other than osteoarthritis.

The study groups were: ***Control group [Group I]:*** included 25 apparently healthy volunteers. They were approved with laboratory investigations and they were 13 females and 12 males. ***Patients group [Group II]:*** included 50 patients with early KOA; they were 31 females and 19 males.

The patients included in this study were subjected to the following: full medical history taking, general examination with weight, height measurement and BMI calculation. Finally, extensive musculoskeletal examination with complete knee joint examination.

US assessment: All patients and controls underwent US examination of the knees by a second blinded radiologist experienced in this technique, with a commercially available ultrasound real-time scanner [Voluson E6 with multi frequency transducer 7-12 MHZ].

Radiographic assessment: weight-bearing

anteroposterior [AP] and lateral knee radiographs were read by expert radiologist, and the severity of OA on the AP view was determined by using the Kellgren and Lawrence [KL] scale [scores 0–4].

Laboratory investigations: MMp-2 was detected by Elisa Kit supplied from [SunRed catalogue No 201-12-0905]. The kit used a double-antibody sandwich enzyme-linked immunosorbent assay [ELISA]. Human Matrix metalloproteinase/ Gelatinase A [MMP2/ Gelatinase A] in samples in the serum was added to monoclonal antibody Enzyme well which was pre-coated with Human Matrix metalloproteinase/ Gelatinase A [MMP2/ Gelatinase A] in samples monoclonal antibody, incubation; then, MMP2/ Gelatinase A antibodies labeled with biotin was added and combined with Streptavidin-HRP to form immune complex; then incubation was carried out and washing again to remove the uncombined enzyme. Then by adding Chromogen Solution A & B, the color of the liquid changes into the blue and at the effect of acid the color finally became yellow. The chroma of coloration and the concentration of MMP2/ Gelatinase A of samples have been positively correlated[16].

Statistical analysis:

Data were analyzed using Statistical Package for Social Science [SPSS] version 19 [IBM®SPSS® Incl, USA]. Quantitative data were expressed as mean ± standard deviation [SD]. Qualitative data were expressed as frequency and percentage. The following tests were done: Chi-square test [X^2] was used to compare non-parametric data. Independent *t*-test was used to compare between two means. Probability [P-value], P-value <0.05 was considered significant.

RESUTLS

In the present study, there is significant difference between the two groups regarding family history. High prevalence of KOA is observed in older patients who also have positive family history with the disease. Otherwise, no statistically significant differences are estimated between the studied groups regarding sex distribution [patients' group: 19 males, control group: 12 males; p=0.65] and other demographic data [Table 1].

According to the Knee manifestations of OA detected by MUS, the majority of patients [40% and 44%] have mild osteophyte on left and right respectively [figures1 & 2], and only one patient has Backer cyst on right, and another one patient have Backer Cyst on left. In addition, cartilage thickness grade-1 and 2 was founded in nine and one patient respectively [Table 2].

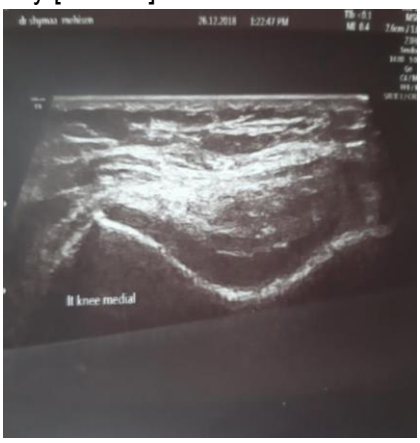

Figure [1]: Transverse view of musculoskeletal ultrasound of left knee showing Medium osteophyte

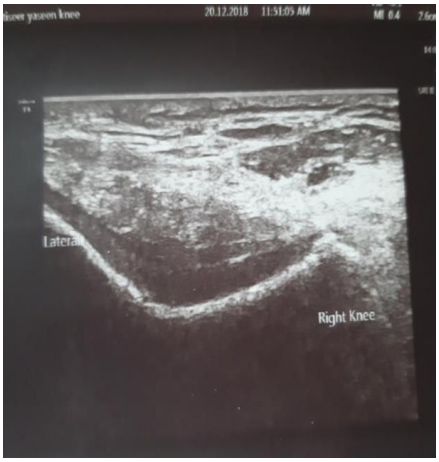

Figure [2]: Transverse view of musculoskeletal ultrasound of right knee showing Cartilage thickness grade 2a and osteophyte

In table [3], there was significant difference between knee OA patients' group and control group as regard to patient weight and BMI. In addition, MMP2 was highly expressed in patients group compared to control group.

From table [4], our data clarified that significant differences are found between the studied groups according to plain X- ray. The majority of patients had joint space narrowing especially from the sky view. In addition, higher MMP-2 expression was found in KOA patients with narrowing view than normal view. Table [5] showed the correlation of MMP-2 expression with other studied parameters and revealed that, there was mild, statistically significant positive correlation between MMP-2 expression with each of weight and BMI.

Table [1] Demographic data of the studied patients' groups.

		Control group **N=25**	***Patients group*** **N=50**	p
Age [years]	Mean ± SD	32.24±2.84	35.08±3.87	0.45
Sex [n,%]	Male	12[48.0]	19[38.0]	0.407
	Female	13[52.0]	31[62.0]	
Residence [n,%]	Urban	14 [56.0]	22 [44.0]	0.326
	Rural	11 [44.0]	28 [56.0]	
Smoking [n,%]		3 [12.0]	10 [20.0]	0.524
Positive Family history[n,%]		3[12.0]	27 [54.0]	0.001*
Hypertension [n,%]		4[16.0]	7 [6.0]	0.067

NB: [*] indicates statistical significant difference

Table [2]: MUS finding in OA patients' group.

Knee manifestation	**N**	**%**
Cartilage thickness Grade 1	9	18
Cartilage thickness Grade 2	1	2
Mild osteophyte on the left	20	40
Mild osteophyte on the right	22	44
Backer Cyst on the left	1	2.0
Backer Cyst on the right	1	2.0

NB: [*] indicates statistical significant difference

Table [3]: Relation between final results and age & surgery time among the studied cases

	Control group **N=25**	***Patients group*** **N=50**	p
Weight [kg]	72.3±6.29	91.86±9.87	<0.0.001*
Height[m]	1.68±0.027	1,7±0.028	0.0.103
BMI[kg/m^2]	25.3± 1.86	31.86 ±3.51	0.001*
MMP-2	12.7±4.29	81.59±19.8	0.001*

NB: [*] indicates statistical significant difference

Table [4]: Comparison of MMP-2 expression in the studied groups regarding to plain X- ray AP view

Matrix metalloproteinase						
		Control group N=25		Patients group N=50		p
		Mean	SD	Mean	SD	
Plan X-ray AP view	Normal	17.2	3.17	84.73	15.2	<0.001*
	Narrowing	8.2	1.12	87.44	17.8	
Sky view	Normal	25.4	3.37	11.7	16.06	<0.0001*
	Narrowing	9.2	1.11	81.83	15.68	

NB: [*] indicates statistical significant difference

Table [5]: Correlation of MMP-2 expression with other studied parameters

	MMP-2 expression	
	r	P
Weight	0.244	0.043*
Height	-0.062	0.333
BMI	0.252	0.038*

NB: [*] indicates statistical significant difference

DISUCSSION

Osteoarthritis [OA] is a critical health problem which considered the most common disease of the joints and the most common chronic disease of the elderly, causing pain and disability which significantly affect the quality of life[17]**.** Therefore, recently much researches deal with the clinical impact of expression of different proteins as biomarkers and/or their effects on the KOA. So, the current study has been tried to highlight the role of MUS and MMP2 in the diagnosis of early KOA.

In the current study, hypertension was found to be increased in cases with KOA when compared to controls, but did not reach statically significance. So, monitoring blood pressure may be warranted among

people with KOA to prevent its consequences [18].

The Knee OA patients revealed variable manifestations on MUS and results of the current work coincides with the study of Oyamakinde et al. who reported that, the most common US finding was osteophytes; in which tibial and femoral osteophytes were seen in 67.4% and 66.5% respectively. This is possibly because most OA patients recruited into that study had K–L radiographic grades II-IV. Also, 37% of participants have osteophytes[19]. Our findings are comparable to previous two studies of Esen et al. and Wu et al.[20, 21]. The prevalence of osteophytes in their studies was 100% and 88%, respectively. The higher prevalence rate recorded in Esen et al. study may be due to their smaller sample size, as only 15 patients with knee OA were examined[20].

Regarding to MMP-2 expression, results of the current work revealed that, the KOA patients have significantly higher MMP-2 concentration when compared to control group. Likewise, Lipari and Gerbino revealed high levels of MMP-2 and MMP-9 in OA and suggested that MMPs may contribute to the cartilage destruction in OA[22]. The meta-analysis of Zeng et al. examined the relationship between the expression of MMP-1, MMP-2, and MMP-9 proteins and the pathogenesis of OA reported that the protein levels of MMP-2 was higher in patients with OA than those in the control group [23]. Burrage and Brinckerhoff study clarified that, serum MMPs levels are related to OA stages and MMP-2 and MMP-13 have been found in active forms[24]. They are particularly significant in the progression and severity of osteoarthritis regardless of etiology[25].

Our study was extended to evaluate the correlation between MMP-2 and other parameters. Patients with narrowing view have increased MMP-2 expression compared to patients with normal view. In addition, there was a significant correlation between MMP-2 expressions with weight and BMI. This is partially agree with Pajak et al. study who reported a significant correlation between MMP-2 and weight [26].

The strength of the present work is the combination between MUS and MMP-2 to detect early KOA. This combination is unique, MUS can detect minimal changes, even in the soft tissues and MMP-2 could confirm the MUS findings early in the course of the disease and both could be used to follow up the response to treatment. This could halt the progress of the disease or at least slows its progression.

Conclusion:

MMP-2 as diagnostic biomarkers ideally identify patients at the early stage of OA where treatment may be most effective. However, no single biomarker stands out alone for use in diagnosis. MUS is an excellent and inexpensive imaging technique to detect synovitis and early OA, and this has offered the grounds to establish its association with symptom flares and in the long term follow up.

Financial and Non-Financial Relationships and Activities of Interest

None to disclose

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