

Estimation of serum hyaluronic acid (HA) in newly diagnosed patients with osteoarthritis of the knee

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تقدير كمية حامض الهيلورونك في مصل مرضى هشاشة العظام حديثي التشخيص

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المستخلص

الهدف من الدراسة: قياس مستوى حامض الهيلورونيك في مرضى هشاشة العظام (OA) حديثي التشخيص مع مقارنتها بوجود ضوابط صحية طبيعية. تصميم: دراسة لحديثي تشخيص لمرضى هشاشة العظام (OA).
مكان العمل: مستشفى الكرامة التعليمي في مدينة الكوت، العراق.
المرضى والطريقة: هذه الدراسة، جميع المرضى خضعوا لجراحة العظام (أكتوبر 2013 إلى سبتمبر 2014) في المستشفى التعليمي الكرامة في مدينة الكوت
المرضى: تم تقسيم العينات إلى مجموعتين، المجموعة الاولى (40) التشخيص حديثا لمرضى هشاشة العظام (OA) المرض) ومجموعة الثانية (من تتكون (25) متطوع اعتيادي (كمجموعة تحكم صحيح)). كان هناك 27 من الذكور و 13 من الإناث المرضى. جميع المرضى وفق المعايير المرضيه التالية: (1) في الفئة العمرية 45-65 سنة؛ (2) لديها آلام في الركبة استمرت لمدة شهر واحد على الأقل، من دون علاج (3) التشخيص بمساعدة الأشعة السينيه المتخذة للركبة خلال شهر واحد ؛ (4) طبيعي النشاط أي ليس طريح الفراش (5) ليس هناك اي تغييرات في الأحذية أو أجهزة لتقويم العظام خلال فترة الدراسة. بالإضافة إلى مجموعة الثانية التي تتكون من متطوعين أصحاء ((25 شخصا، ومطابقة العمر والجنس، اتخذت كمجموعة تحكم. (6) جميع المرضى الذين يعانون من متوسط ESR اكثر من 39mm/hr .
الاستنتاجات: هناك ارتفاع ملحوظ في مستوى حمض الهيلورونيك ($P < 0.0005$) في تشخيص المرضى الذين يعانون من هشاشة العظام (حديث التشخيص) حيث يمكن أن تستخدم الباراميتير للتشخيص للمرض (خصوصية 40%، 92% حساسية باستخدام 0.37ملغ /مل كحد قاطع قيمة وخصوصية 93%، والحساسية 52% باستخدام 1.5نانوغرام /مل كحد قاطع.

Abstract

The objective of the study: measurement the level of hyaluronic acid in patient with newly diagnosis of osteoarthritis (OA) and compared them with normal healthy controls.

Design: Single-institution study of patients with newly diagnosis of osteoarthritis (OA).

Setting: Al- Karama Teaching Hospital in Al- Kut city, Iraq

Patients: This study incorporated all patients underwent Orthopaedic (October 2013 to September 2014) in Al- Karama Teaching Hospital in Al- Kut city.

The patients were divided into two groups, group 1(40 newly diagnosis patients with osteoarthritis (OA) disease) and group 2 (25 normal healthy controls). There were 27 male and 13 female patients. All patients fulfilled the following inclusion criteria: (1) an age range 45-65 years; (2) have knee pain that had lasted for at least one month, without treatment(3) knee radiographs taken within one months; ; (4) a normal activity level—i.e.,not bedridden (5) no changes in shoes or orthotic devices during the study period. In addition to group 2 that consist of healthy volunteers (25 persons), age and sex matched, were taken as control group (6). All patients with ESR average more than39mm/hr.

Introduction

Hyaluronic acid appears to be a constituent of the intracellular cement of all tissues. This material is present in high concentration in the umbilical cord, synovial fluid, and in the vitreous humor of the eye. It has been recently reported in free form in several pathological human sera (1, 3) and in proteolytic digests of normal human plasma (2). Knee osteoarthritis (OA) is a major public health problem across the world. Population based studies revealed that symptomatic knee OA is present in 20-30% of the elderly population aged >65 years (4, 5) and its prevalence is increasing due in part to the aging of the population (6). Clinical symptoms are dominated by chronic knee joint pain, which leads to disability, psychological distress, and impaired quality of life. Recently, patient-oriented outcome measures have been acquiring greater importance in treating knee OA (7). The current treatment for knee OA consists of conservative treatment, such as exercise, physical therapy, pharmacological agents and, in some cases, surgical treatment (8, 9). While many of the commonly used conservative treatments have been recognized to be effective^[10], there is still insufficient evidence available. Among the pharmacological treatments for knee OA, oral non-steroidal anti-inflammatory drugs (NSAIDs) act rapidly and are recommended for the management of OA. Although frequent and serious adverse effects of NSAIDs have been recognized (10).

The present study aimed to

- 1- Estimate and compared the serum hyaluronic acid level in normal healthy controls and OA patients.
- 2- The possibility of using serum HA levels as diagnostic parameter in the diagnosis the patients with OA.

Subjects and methods

All common laboratory chemicals and reagents used in this study were of analar grade unless otherwise specified and were obtained from the following companies: Cusabio company; Hyaluronic acid, Sodium citrate (UK), Biotek (USA); Elisa washer, Biotek (USA); Elisa reader and ESR rack (UK).

Determination of hyaluronic acid (HA)

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for HA has been pre-coated onto a micro plate. Standards and samples are pipetted into the wells and any HA present is bound by the immobilized antibody. After removing any unbound substances, a biotin- conjugated antibody specific for HA is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells, following a wash to remove any unbound avidin- enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of HA bound in the initial step. The color development is stopped and the intensity of the color is measured.(according to the sheet user manual :-cusabio company).

Statistical analysis

Student's t-test was used to determine if the mean value for biochemical tests were significantly different in the normal healthy control and OA patient, $p < 0.0005$ were considered significant.

Results

The results obtained from this study showed that HA level is increased significantly ($P < 0.0005$) in patients with Knee osteoarthritis (OA) when compared with normal healthy control (1.5 ± 1.422 , 0.37 ± 0.217) respectively as shown in table (1) and figure (1). This increased level was significant ($P < 0.0005$) which may be used as diagnostic parameter of this disease. The predictive values of serum HA level in OA patients as compared with normal healthy controls were done by using two cut-off, one at 0.37ng/ml (calculations were carried out by taking the mean of normal healthy controls) and the second at 1.5 ng/ml (calculations were carried out by taking the mean of OA patients). Sensitivity, specificity, predictive values of positive and negative tests and efficiency test of serum HA were considered in table (2) using 0.3 ng/ml as cut-off value. Test sensitivity was 92%, test specificity was 40%, and efficiency 73%. Positive predictability and negative predictability were considered in table (2). By using 1.5 ng/ml as cut-off value the specificity was increase to 93% and sensitivity and efficiency were decreased to (52% and 66% respectively) as shown in Table (3). The ESR results showed that 19% of patients have ESR within normal range less than 20 mm/hr and 81% have ESR between 30 ---80 mm/hr. Figure (2) showed the negative correlation between Hyaluronic acid (HA) concentration and ESR values to the same patients.

Table (1): Biostatistical calculations and student t-test for serum HA in sera of normal healthy controls and OA patients

HA ng/ml	Normal healthy controls	Knee OA
Sample size	N1=25	N2=40
Means ng/ml	X1=0.39	X2=1.5
Standard deviation	SD1=0.217	SD2=1.422
Standard error of the mean	Sx1=0.0447	Sx2=0.225
Confidence interval of the mean	0.129—0.305	0.972—1.87
t-test		3.933
Probability		P<0.0005

*Calculation based on unpaired observations of serum HA level (ng/ml) for normal healthy controls, and osteoarthritis (OA) patients .

* t-test and probability for osteoarthritis (OA) patients as compared to normal healthy controls.

Table (2): Predictive value of Hyaluronic acid level in OA patients using 0.37ng/ml as cut-off value

Sensitivity *	92 %
Specificity **	40%
Positive predictability ***	73%
Negative predictability ****	75%
Efficiency &	73%

*Sensitivity =Positive in disease , expressed as percent.

**Specificity =Negative in healthy, or absence of particular disease, expressed as percent.

***Predictive value of positive test=Percent of patients with positive test results that are disease.

**** Predictive value of negative test = Precent of patients with negative test results that are non disease.

& Efficiency test = Percent of patients correctly classified as diseased and non diseased.

Table (3): Predictive value of Hyaluronic acid level in OA patients using 1.5 ng/ml as cut-off value

Sensitivity *	52 %
Specificity **	93%
Positive predictability ***	93.3%
Negative predictability ****	53%
Efficiency &	66%

*Sensitivity =Positive in disease , expressed as percent.

**Specificity =Negative in healthy, or absence of particular disease, expressed as percent.

***Predictive value of positive test=Percent of patients with positive test results that are disease.

**** Predictive value of negative test = Percent of patients with negative test results that are non disease.

& Efficiency test = Percent of patients correctly classified as diseased and non diseased.

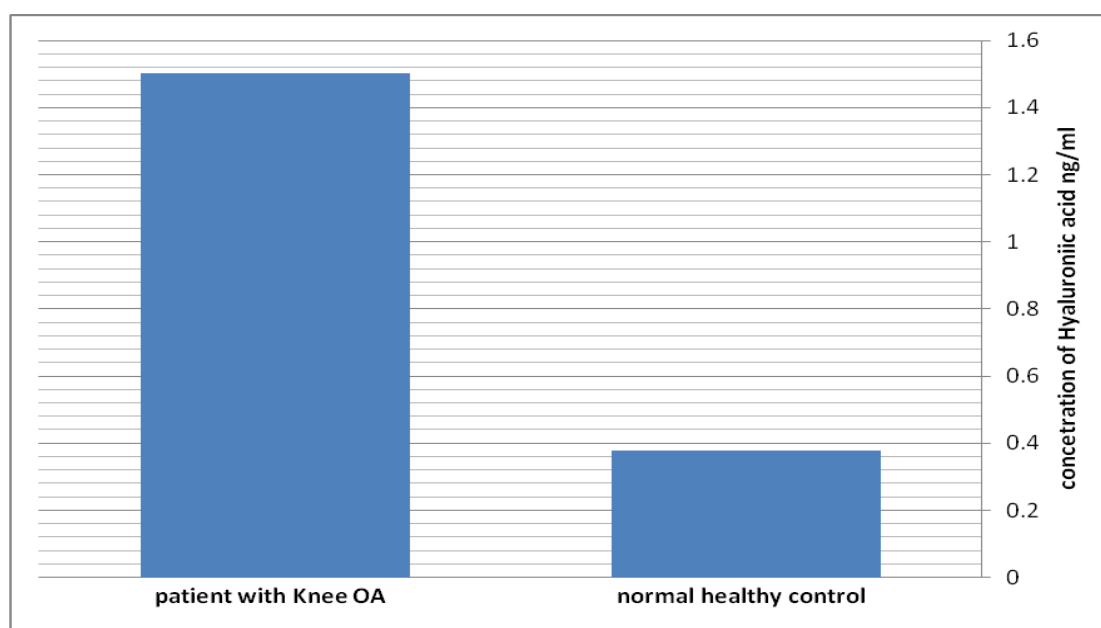


Figure (1):Serum HA in sera of normal healthy controls and patients with osteoarthritis (OA) (mean \pm SD)

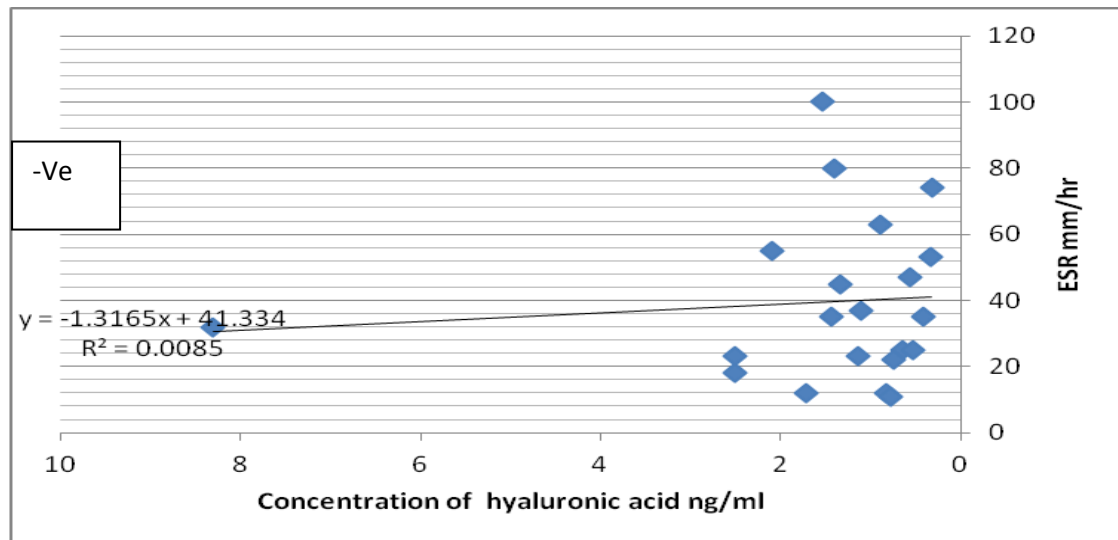


Figure (2): HA values and corresponding ESR individual for the same samples from patient with osteoarthritis (OA)

Discussion

The change in serum HA level are characteristic of many pathological conditions (3) including OA. The results of the study revealed highly significant elevation of HA in the OA patients ($p < 0.0005$). There was significant elevation in the ESR value of the patient as compared with the normal healthy control. The diagnostic validity of HA in OA patients compared to that normal healthy control 0.37 ng/ml (average of normal healthy controls) as a cutoff value (specificity 40%, sensitivity 93%) as shown in table (2), and table (3) showed the diagnostic validity by using 1.5 ng/ml (average of OA patients) as a cut off value(specificity 93%, sensitivity 52%). The increase in specificity from 40% to 93% and decrease in sensitivity from 92% to 52% using cut-off value 1.5 ng/ml is to eliminate the moderate increase of HA in OA , so that we can differentiate the non- osteoarthritis disease from normal healthy controls by using HA level. Table (1) and Figure (1) showed the mean \pm SD of HA level in patient with the OA. The increase level of HA in sera of patient with OA may be due to leakage of HA from the breakdown of cartilage due to the disease condition. Future studies will analysis HA from both synovial fluid and serum to precisely or accurately whether serum HA is a good matter in osteoarthritis OA diagnosis. Our results in agreement with that reported by Suzuki (11), and disagreement with J.A.Marsh (12).

Conclusions

There is a significant rise in the level of Hyaluronic acid($P < 0.0005$) in the newly diagnosis patients with osteoarthritis that it can be used as diagnostic parameter of the disease (specificity 40%, sensitivity 92% by using 0.37 ng/ml as cut-off value and specificity 93%,sensitivity 52% by using 1.5 ng/ml as cut-off value) .

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