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Assessment of Serum and Salivary Vimentin Levels in Rheumatoid Arthritis Patients

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ABSTRACT

Aim: This work aimed to determine the serum and salivary level of vimentin in patient with RA.
Materials and Methods: A total of 84 participants with RA (69) and controls (15) were submitted in this work. Serum and Salivary vimentin levels and other clinical measures in serum [Anti Cyclical Citrullinated Peptide Antibody (ACCP), Rheumatoid Factor (RF)] were determined by quantitative enzyme-linked immunosorbent assay (ELISA).
The sensitivity and specificity of vimentin was determined by Receiver-operating characteristic (ROC) curves analysis. The relationship between serum and salivary level of vimentin in RA patients with other clinical measures was assessed by Spearman's rank correlation coefficient.
Results: (Mean ± SD; Median) of serum vimentin concentration (mg/ml) in RA patients was (21.974 ± 12.639; 23.096) which was significantly higher than in healthy subjects (6.704 ± 2.720; 6.031; P<0.001). Patients with RA and controls were compared by using ROC curve analysis, showed a significant (P<0.001); ROC curve showed that area under the curve (AUC) of 0.929 (95% confidence interval: 0.869-0.990). At a cut-off of 6.5 mg/mL, serum vimentin had a sensitivity of 91.3% and specificity of 80%. (Mean ± SD; Median) of salivary vimentin concentration (mg/ml) in RA patients (8.059 ± 4.943; 6.295) was higher than that in healthy subjects (6.436 ± 4.477; 5.232) with no significant result statistically. ROC was drawn to make comparison between RA patients with controls, showed a significant (P<0.001); ROC curve showed that area under the curve (AUC) of 0.918 (95% confidence interval: 0.0794–1.000). At a cut-off of 5.5 mg/mL, salivary vimentin had a sensitivity of 98.6% and specificity of 60 %.
The results show levels of salivary vimentin was no significantly correlated with levels of serum vimentin. However, serum vimentin positively correlated with serum anti cyclic Citrullinated peptide (ACCP) (r=0.522, P<0.001) and serum rheumatoid factor (RF) (r=0.430, P<0.001), but no significant result with 28-joint disease activity score (DAS) in patients with RA.
Conclusions: Vimentin in serum is useful than salivary vimentin to detect patient with rheumatoid arthritis.
Key words:
Vimentin, Rheumatoid arthritis, DAS28, Anti cyclical citrullinated peptide antibody, Rheumatoid factor

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INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune chronic inflammatory disease features symmetrical small joints inflammation and affects approximate 1% of the population all over the world [1]. If RA patients left without treatment leads to severe joint destruction resulting in impaired physical function and disability in the workplace [2].

There are multiple autoantibodies that have been reported detectable in the serum of patients, which are associated with RA. ACCP antibodies and Rheumatoid factors have proven to be useful diagnostic tools especially in the early stages of disease and for predicting disease progression [3].

Furthermore, disease activity score 28 (DAS28) is used to assess disease course and treatment outcome and is based on the number of 28 joints identified for swelling and pain [4]. However, the manifestations of RA are too diversified for physicians to distinguish those not that well differentiated from patients suffering from other diseases sharing common clinical features, resulting in many patients, earlier-staged and/or serum-negative patients in particular, being missed or missed diagnosed.

Therefore, researchers are working on finding novel diagnostic and predictive biomarkers with higher sensitivity and specificity to improve the diagnostic accuracy and disease outcome of RA [5].

Vimentin is a protein of intermediate filament family, which is expressed in all mesenchymal cells. Vimentin plays a key role in the physiology of the cell, cellular interactions and the functioning of the immune system. Vimentin is one of the antigens involved in the pathogenesis of rheumatoid arthritis (RA) [6].

In spite of vimentin is a cytoplasmic protein predominantly, it can be present at extracellular locations, either at the surface of many cell types or in a secreted form, often in relation to cell activation, injury, inflammation or senescence, citrullination is an important modification of extracellular vimentin, it can occur under any condition, physiological or pathological. Vimentin turns into an antigen by peptidyl arginine deiminases enzyme in rheumatoid arthritis [7].

Multiple proteoforms of extracellular vimentin, whether exposed on the cell surface or secreted, may perform complicated and potentially double-edged effects through interactions with various receptors or targets, in a context-dependent way, as summarized above. Knowledge vimentin's role in pathogenesis will need a better understanding of these mechanisms.

The sensitivity and specificity of ACCP in RA and healthy individuals were determined using a quantitative enzyme-linked immunosorbent assay in this study. In addition, serum and salivary vimentin were studied in RA patients and healthy individuals to see their benefit.

PATIENTS AND METHODS

The patients were allocated from rheumatology consulting clinic in Baghdad teaching hospital. Demographic evaluation was done for all participants (Table 1). A total number of 84 subjects were enrolled in this study, divided into groups:

- Group I (control group): The number of the participants was 15 (2males and 12 females). Their median age was 43 years, with inter-quarter (Q1-Q3) 40-45, 6 of participants were ≤40 years and 9 of participants were >40 years.
- Group II (RA patients): The number of the participants was 69 (14males and 55 females). Their median age was 47 years, with inter-quarter (Q1-Q3) 40-45, 19 of participants were ≤40 years and 50 of participants were >40 years.

There was no significant difference in age and gender between the two groups.

The criteria of inclusion were: patients with newly diagnosed or known cases of patients with rheumatoid arthritis. The criteria of exclusion included: patient with diabetes mellitus, pregnancy and lactated mothers, malignancy, chronic liver disease, end-stage renal failure and other autoimmune diseases.

Sixty-nine patients with RA in this study were subjected according to criteria of American College of Rheumatology (ACR) in 2010 (8), then measurement of serum and salivary vimentin levels were assessed. ACPA and RF were recorded in serum as well as clinical and demographic data were obtained. Ethical approval for all participants was obtained before the work.

Collection of saliva samples

From each participant the whole un-stimulated mixed (resting) saliva was obtained. A two-hour fast was required before collection. Individual rinsed their mouth with tap water.

The saliva samples were collected into disposable containers. Then centrifuged (3000rpm) for 10 min. The volume of each saliva sample was measured after separating the supernatants, then the samples were kept in an Eppendorf tube and frozen at (-20°C) to be used for biochemical analysis.

Table 1: Demographic and clinical characteristics of the participants.

	Group I (n=15)	Group II (n=69)
Sex (Male: Female)	2:13	14:55
Age (year)		
Median	43	47
Inter-quarter (Q1-Q3)	40-45	40-45
≤ 40	6	19
>40	9	50
Duration of disease (year)		
<1		15 (21.8)
5-Jan		27 (39.1)
>5		27 (39.1)
Status of Disease activity		
Active (relapse)		59 (85.5)
Inactive (remission)		10 (14.5)

DAS-28 Score	
Remission (n=10)	2.28±0.16
Low (n=11)	3.00±0.14
Intermediate (n=45)	4.02±0.49
High (n=3)	5.92±0.10

The results expressed as number (%), median, inter-quartile, mean ± SD. P-value for categorized data was calculated by using Chi-squared test for comparison between Groups I and II Group I: control participants, Group II: rheumatoid arthritis patients, DAS: disease activity score.

Immunoassays of serum vimentin, RF, ACCP and salivary vimentin

Serum and salivary concentrations of vimentin in RA patients and controls were measured by ELISA using reagents supplied by Bioassay Technology Laboratory (china). ACCP was measured by ELISA using reagents supplied by Bioassay Technology Laboratory (England).

RF was measured by ELISA using reagents supplied by CELL BIOLABS, INC. ELISA tests were performed according to the manufacturer's instructions.

Statistical analysis

The results expressed as number (%), median, inter-quartile, mean ± SD. P-value for categorized data was calculated by using Chi-squared test for comparison between Groups I and II Group I: control participants, Group II: rheumatoid arthritis patients, DAS: disease activity score.

The results of assessment of biomarkers related to rheumatoid arthritis compared with health subjects are expressed as mean ± SD (median). P-value was calculated using independent two sample t-test for continuous data. Receiver-operating characteristic (ROC) curves analysis was performed to determine the sensitivity and specificity of serum and salivary vimentin.

The relationship between serum and salivary vimentin measured then the relationship of serum vimentin and other measurements in patients with RA was assessed using Spearman's rank correlation coefficient. Statistical analysis was performed with SPSS software (version 24.0). P value <0.05 denoted statistical significance.

RESULTS

Serum vimentin concentration (mg/ml) in patients with RA (Mean ± SD; Median) (21.974 ± 12.639; 23.96) was significantly higher (P<0.001) as compared with that in healthy subjects (6.704 ± 2.720; 6.031).

Salivary vimentin concentration (mg/ml) in RA patients (8.059 ± 4.943; 6.295) was higher than that in healthy subjects (6.436 ± 4.477; 5.232) with no significant result statistically.

RF and ACCP levels in RA patients was significantly higher (P<0.001) compared with healthy subjects as shown in (Table 2). In order to determine the sensitivity and specificity of serum vimentin. ROC analysis was performed comparing results between RA patients and healthy subjects.

ROC curve showed that the (AUC) of 0.929 (95% confidence interval: 0.869 - 0.990). (P<0.001) as shown in (Figure 1). At a cut-off of 6.5 mg/mL, the ROC curve yielded a sensitivity of 91.3% and specificity of 80 %, The Positive predictive value was 95.5 %, Negative predictive value was 66.7% (Table 3).

In order to determine the sensitivity and specificity of serum vimentin. ROC analysis was performed comparing results between RA patients and healthy subjects.

ROC curve showed that the (AUC) 0.918 (95% confidence interval: 0.0794 – 1.000). (P<0.001) as shown in (Figure 1).

At a cut-off of 5.5 mg/mL, the ROC curve yielded a sensitivity of 98.6% and specificity of 60 %. The Positive predictive value was 91 %, Negative predictive value was 81 % (Table 3).

While ROC curve analysis of ACCP in serum comparing patients with RA with controls demonstrated a significant (P<0.001) area under the curve (AUC) of 0.992 (95% confidence interval: 0.979 – 1.000).

At a cut-off of 5.5 mg/mL, the ROC curve yielded a sensitivity of 98.5% and specificity of 80 %, The Positive predictive value was 95.7 %, Negative predictive value was 92.3%.

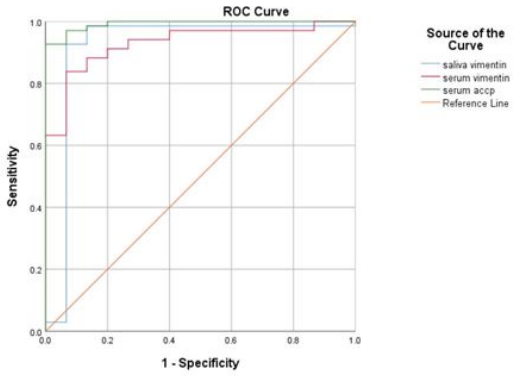
Table 4 displays the correlations of serum vimentin concentration with clinical and serological variables in patients with RA.

The results show levels of salivary vimentin was not significantly correlated with levels of serum vimentin, where the results of correlation analyses revealed that serum vimentin positively correlated with ACCP (r=0.522, P<0.001) and RF(r=0.430, P<0.001) but no significant result with 28-joint disease activity score (DAS) in patients with RA.

Table 2: Assessment of biomarkers related to rheumatoid arthritis compared with health subjects.

	Group I (n=15)	Group II (n=69)	p-value
Salivary vimentin (mg/ml)	6.436±4.477 (5.232)	8.059±4.943 (6.295)	0.226
Serum vimentin (mg/ml)	6.704±2.720 (6.031)	21.974±12.639 (23.96)	<0.001
Serum Anti-citrullinated cyclic peptide (mg/ml)	4.111±1.310 (3.622)	10.012±4.714 (8.349)	<0.001
Serum Rheumatoid factor(IU/mL)	6.931±1.945 (6.945)	15.425±9.845 (12.126)	<0.001

The results are expressed as mean ± SD (median). P-value was calculated using independent two sample t-test for continuous data.



Variable(s)	Area	p-value	95% Confidence Interval	
			Lower Bound	Upper Bound
Serum vimentin	0.929	0.000	0.869	0.990
Saliva vimentin	0.918	0.000	0.794	1.000
Serum ACCP	0.992	0.000	0.979	1.000

Figure 1: The area under the curve of the cut values of vimentin and ACCP in the biological fluids of rheumatoid arthritis patients.

Table 3: The sensitivity, specificity, positive predictive value, and negative predictive value of the biological fluids levels of vimentin and ACCP.

	Cutoff value (mg/ml)	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Serum vimentin	6.5	91.3	80	95.5	66.7
Saliva vimentin	5.5	98.6	60	91	81
Serum ACCP	5.5	98.5	80	95.7	92.3

The results are expressed as percentages.

Table 4: Correlation coefficients (r) of vimentin with clinical and serological measures in patients with RA.

Determinants	DAS	Vimentin	ACCP
DAS			
vimentin	0.142 (0.246)		
ACCP	0.261 (0.030)	0.522 (<0.001)	
Rheumatoid factor	0.242 (0.045)	0.43 (<0.001)	0.463 (<0.001)
Salivary vimentin		0.053 (0.668)	

The results are expressed as Spearman rho correlation factor (P-value). ACCP: Anti-citrullinated cyclic peptide.

DISCUSSION

This study compared the levels of salivary vimentin in patients with RA with those of healthy individuals. However, the levels of serum vimentin, RF and ACCP in RA patient were compared with that in healthy subjects.

The present study demonstrated that the levels of serum vimentin were significantly (P<0.001) higher in patients with RA than in healthy individuals, and it was a good predictor for the risk of RA.

However, the level of salivary vimentin in RA patients was higher than that in healthy subjects with no significant result statistically.

Serum RF and ACCP levels in patient with RA were significantly higher (P<0.001) as compared with that in healthy subjects as shown in (Table 2). Consistent with the previous research results.

A meta-analysis included 14 studies, in which the anti-mutated citrulinated antibody (anti-MCV) and ACCP for the RA diagnosis were tested, concluded that there is no difference between the two tests. Thus, the anti-MCV may be a test of second line used in patients suspected of RA, but with ACCP and RF negative [8-10].

Another meta-analysis demonstrates that anti-MCV is more sensitive but less specific, and has lower diagnostic accuracy than ACCP in RA [11], as there is evidence that this antigen (vimentin) is specific in RA.

Other study shows that antigenic properties of vimentin were determined by mutation and citrullination [12]. The citrullination of the antigens perfectly fits the model for the development and chronic nature of RA. They divided the process of autoimmunity in RA into five steps: an innocent inflammation in combination with massive apoptosis or impaired clearance can lead to the elevation of cytosolic Ca2+ concentrations followed by the activation of peptidylarginine deiminase (PAD) and the citrullination of proteins. When citrullinated antigens are presented to T cells, the production of ACPAs is triggered. Immune complexes can be formed if the antigens react with the autoantibodies. These (immune complexes) IC stimulate inflammatory processes and cause a vicious circle of inflammation resulting in joint destruction for years [13].

Meta-analysis study in 2019 considered commercial ELISA kit of mutant vimentin, MCV, has been extensively used in clinics with a sensitivity of 0.64–0.84 and a specificity of 0.79–0.96 [14]; and this agree with our results that the serum vimentin has sensitivity of 91.3% and a specificity of 80%.

ACCP were the most valuable in the diagnosis of RA; their results ACCP the highest specificity (94.5%). The areas under the curve (AUC) of ACCP was 0.89 (P < 0.01) [15]; which appeared comparable to our results regarding ACCP in Figure 1, the areas under the curve (AUC) of ACCP was 0.992 (P<0.001) indicating a high level of overall accuracy.

In this study, ROC curve at cut-off presented in (Table 3) indicated the specificity of serum vimentin was the same percentage of serum ACCP 80%, the sensitivities of both assays was comparable (91.3%, 98.5% respectively)

While assay of vimentin in saliva had lower specificity in comparison to the ACCP assay despite the great expectations, salivary vimentin assay did not provide additional benefit value over the already established ACCP assay, although credited with higher sensitivity 98.6%;

In addition, our study display correlation analyses between salivary vimentin, serum vimentin, ACCP and/or RF in the diagnosis of RF, The results show levels of salivary vimentin was not significantly correlated with levels of serum vimentin, where the results of correlation analyses revealed that serum vimentin positively correlated with ACCP (r=0.522, P < 0.001) and RF(r=0.430, P<0.001) but no significant result with 28-joint disease activity score (DAS) in patients with RA (Table 4).

A recent study in 2021 show that there was no significant difference in the levels of salivary citrullinated between patients with RA and controls. Thus, salivary citrullinated levels are not associated with RA disease severity. The production of citrullinated proteins could be the result of the daily regeneration of oral tissues who releases their products to the saliva [16].

Some objective factors, such as special geographic location, environment, climate, ethnicity, treatment condition, and disease severity, might introduce heterogeneity between different records. Thus, further research is needed to clarify these issues.

CONCLUSIONS

The ACCP with a high specificity and sensitivity are considered the most meaningful serological biomarkers of RA than vimentin. Further studies performed on a larger number of patients over longer time periods are necessary to confirm this assumption.

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