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Original Article

Assessment of Serum Level of Chemerin in Rheumatoid Arthritis Patients

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ABSTRACT

Background: Chemerin functions as a chemoattractant for immune cells, promotes both innate and acquired immunity, and modulates the activity of immune cells through its interaction with the ChemR23 receptor.

Aim of the work: The objective of this work was to find out the relation between serum level of Chemerin and occurrence of rheumatoid arthritis Disease.

Patients and methods: Sixty patients with rheumatoid arthritis [RA] which divided to 30 early RA and 30 late RA were included and were compared with 30 normal subjects as a control group. All were subjected to full history taking, clinical examination (general and musculoskeletal examination), calculation of body mass index and disease activity by disease activity score and health associated quality of life. This followed by laboratory investigations (mainly Chemerin (ng/ml).

Results: There was a higher significant value of DAS CRP in early [5.45±1.28] than the Late Group [4.29±1.13]. In addition, there was a significant increase of HAQ in early than the late group [1.83±0.52 vs 1.15±0.33]. There was higher mean value of Chemerin [ng/ml] in early than the late group [389.21±112.79 vs 235.05±9.15, respectively], the lowest value was in the control group [169.86±27.52]. Furthermore, there was a significant positive correlation between Chemerin with ESR, CRP, DASCRP and HAQ; while there was a significant negative correlation between Chemerin with duration of disease. The ROC curve showed that the best cutoff of Chemerin "ng/ml" was ≥218.38, with sensitivity of 100% specificity of 100% positive predictive value [PPV] of 100%, negative predictive value [NPV] of 100% with diagnostic area under the curve [AUC] of 1.00.

Conclusion: The results highlight the potential value of serum chemerin levels over established indicators ESR and CRP as an effective biomarker for differentiating between Early and Late RA as well as among those who have rheumatoid arthritis and disease-free individuals.

Keywords: Chemerin; Rheumatoid Arthritis; DAS-CRP; HAQ; ESR.

INTRODUCTION

Rheumatoid arthritis [RA] can cause symmetrical peripheral polyarthritis in the hands and/or feet, which can be debilitating over time, when left untreated [1]. Environmental and genetic variables have been identified in certain cases of RA, however research on the etiology of the disorder is still early [2]. Small peripheral joints, primarily in the hands, are affected by RA. The cervical spine is the only portion of the spine where the axial joints may be affected because synovial joints are located there. The lumbar spine is spared [3].

Although it is common for patients to have many small joint involvements, some may also present with monoarticular and extra-articular involvement [lung and heart] [4]. Adipocytokines that includes adipokines, cytokines, chemokines, and complementing factors are associated with several physiological and pathological processes, because they control blood pressure, glucose and lipid metabolism, immune response, and inflammation [5]. Adipokines play vital roles in rheumatic disorders [local or systemic] and are not only produced by white adipose tissue but also by immune cells, chondrocytes, and synoviocytes [6].

Chemerin is one of adipokines that is encoded by the human 7q36.1 retinoic acid receptor responder protein 2 [RARRES2] gene, also known as tazarotene-induced gene 2 [TIG2] [7]. Chemerin contributes to immune responses by acting as a chemoattractant for immune cells, facilitating innate and acquired immunity, and interacting with the ChemR23 receptor to regulate immune cell behavior [8]. Chemerin plays a significant role in inflammation by recruiting inflammatory cells, contributing to vascular remodeling, and regulating the production of pro-inflammatory cytokines. Its involvement in the inflammatory response suggests its potential as a target for therapeutic interventions in diseases like COPD [9]. Therefore, the current study aims to determine the level of serum Chemerin, its correlation with RA patients, and the feasibility of using it as a future RA diagnostic tool.

PATIENTS AND METHODS

A total of 60 patients with rheumatoid arthritis and **30** normal subjects were enrolled in this case-control study and collected from the outpatient and inpatients clinics of Al Hussein and Bab El Sharia Hospitals of Al Azhar University - Cairo. And subdivided into three groups: Group A: 30 patients with early rheumatoid arthritis. Group B: 30 patients with late [chronic] rheumatoid arthritis. Group C: 30 healthy age-matched individuals [Control group].

Inclusion criteria and Exclusion criteria: Sixty patients aged more than 18 years old and less than 60 years old included males and females who were diagnosed according to EULAR\ACR 2010 criteria.[10] and divided to early RA [less than 12 months] and late RA [more than 12 months] [11] were included in our study. 30 normal age- and sex-matched subjects were selected as controls. Participants with any of the following illnesses were excluded from the study: Infection, Malignancy, Hematological disorders, Cardiac disorders, Endocrinal disorders and other rheumatological disorders.

All participants were subjected to collection of data regarding a complete medical history, which includes personal history, past history, history of current illness and review of other systems as well as family history and current medications. In addition, all were submitted to general and musculoskeletal examination; calculation of body mass index [12] and disease activity by DAS 28-CRP [13]; assessment of physical function activity by HAQ [14]; and laboratory investigation.

Blood sample: 9 milliliters of fasting venous blood were drawn and divided into two portions: 3 milliliters went into an EDTA-containing tube for CBC analysis and ESR testing by using Westergren method [as determined by Sysmex XB and Celdyne Ruby, Automated Hematology Analyzer]; 6 milliliters were left to clot; and 6 milliliters were centrifuged to separate the serum and divide it into two portions. CRP and IgM-rheumatoid factor [IgM-RF] using the immune-phelometry method, and anti-CCP antibody using ELISA were determined using the first aliquot [measured by the Cobas C311 auto analyzer utilizing Roch]. Serum TC, TG and UA were measured spectrophotometrically by enzymatic reaction. Serum HDLc was determined after the precipitation of other lipoproteins by the reagent containing sodium phosphotungstate along with MgCl and the cholesterol contents in the supernatant were measured using the cholesterol kit. VLDLc was calculated using the formula TG/2.19, and LDLc was computed from Friedewald's formula: LDLc = TC − HDLc – VLDLc.

The second aliquot was frozen at –20 °C to assess human Chemerin later on. Bioassay Technology Laboratory Company's ELISA kit with Cod E1435Hu intra-assay precision 4.3 was used to measure human Retinoic acid receptors protein 2. This kit performs an enzyme-linked immunosorbent assay [ELISA] and supplied by BT LAB 501Changsheg S Rd, Nanhu Dist, Jiaxing, Zhejiang, China. The human Retinoic acid receptors protein 2 antibody [RARRES2]; Chemerin antibody has been pre-coated on the plate. The addition of RARRES2; Chemerin to the sample causes it to attach to antibodies coated on the wells. A biotinylated human RARRES2; Chemerin was added, and it binds to RARRES2; Chemerin in the sample. The biotinylated Chemerin antibody is then bound by the addition of streptavidin-HRP. Unbound Streptavidin-HRP was removed during a washing step after incubation. Substrate solution was then added, and the color increased in direct proportion to the amount of human RARRES2; Chemerin present. By adding an acidic stop solution, the process was stopped, and absorbance at 450 nm was measured. The range of detection was 0.5–1.5 mg/dl.

Statistical analysis: Recorded data were analyzed using the statistical package for social sciences, version 23.0 [SPSS Inc., Chicago, Illinois, USA]. The quantitative data were presented as mean± standard deviation and ranges when their distribution was parametric [normal] while non-normally distributed variables [non-parametric data] were presented as median with inter-quartile range [IQR]. Also, qualitative variables were presented as number and percentages. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk Test. P-value $\leq$0.05 was considered significant. P-value $\leq$0.001 was considered as highly significant. P-value >0.05 was considered insignificant.

RESULTS

Table [1] showed highly significant higher mean value of DAS CRP in early group [5.45±1.28] than the Late Group [4.29±1.13], p<0.001. There was a significant higher frequency of high disease activity in early group [[90%] comparing to late group [53.3%], p=0.006**. Table [2]** showed highly significant higher mean value of HAQ in early than the late group [1.83±0.52 vs 1.15±0.33, p<0.001]. in addition, there was a s significantly higher frequency of severe disability in early group than the late group [30% vs 0.0%, p<0.001. **Table [3]** showed significantly higher mean value of Chemerin [ng/ml] in early than the late group [389.21±112.79 vs 235.05±9.15, respectively], and the lowest value was in the control group [169.86±27.52]. Also, there was a significantly higher median value of ESR [mm/h] in late group [45 [25.8-62.5], followed by early group [30 [20-46.3], and the lowest value was recorded in the control group [8 [8-12] [p<0.001]. As well as, a highly significant higher medians of CRP were recorded in the late group [12.8 [8.3-42], followed by early group [10.5 [4-17.3], and the lowest value in the control group [2 [0-3] [p<0.001]. **Table [4]** showed a significant positive correlation between

Chemerin "ng/ml" with ESR [mm/h], CRP [mg/L], DAS CRP, HAQ and RF [IU/ml] [p<0.05]; while, there was a significant negative correlation between Chemerin "ng/ml" with duration of disease "months" [p<0.05]. Also, a significant positive correlation was recorded between ESR "mm/h" and Chemerin [ng/ml], CRP m[g/L], age [years], duration of disease [months], RF [IU/ml] and anti CCP [u/ml] [p<0.05]. As well as a significant positive correlation between CRP "mg/L" with Chemerin [ng/ml], ESR [mm/h], RF [IU/ml] and Anti CCP [u/ml] [p<0.05]. **Table [5] and Figure [1]** showed that ROC curve was used to define the best cut off value between early group vs. control Group regarding Chemerin "ng/ml" which was ≥218.38, with sensitivity of 100% specificity of 100% positive predictive value [PPV] of 100%, negative predictive value [NPV] of 100% with diagnostic area under the curve [AUC] of 1.00. While cut off value of ESR was ≥19, with sensitivity of 83.3% specificity of 96.7% PPV of 96.2%, NPV of 85.3% with diagnostic AUC of 0.95. Also as cut off value of CRP was >3, with sensitivity of 80% specificity of 100% positive predictive value of 100%, negative predictive value of 83.3% with diagnostic AUC of 0.91. The good discrimination between Early group vs. control group regarding Chemerin was AUC 1.00 [0.94-1.00], followed by ESR [mm/h] was AUC 0.95 [0.86-0.99], then CRP m [g/L] was AUC 0.91 [0.81-0.97] [p<0.001].

Table [1]: Comparison between groups according to DAS.

		Early Group[n=30]	Late Group[n=30]	Test value	p-value
DAS CRP	Mean±SD	5.45±1.28	4.29±1.13	4.962	<0.001**
	Range	2.1-8.55	1.99-5.96		
DAS CRP Level	Remission disease activity	1[3.3%]	2[6.7%]	10.290	0.006*
	Moderate disease activity	2[6.7%]	12[40.0%]		
	High disease activity	27[90.0%]	16[53.3%]		

Using: t-Independent Sample t-test for Mean±SD; x^2: Chi-square test for Number [%] or Fisher's exact test, when appropriate; p-value >0.05 is insignificant; *p-value <0.05 is significant; **p-value <0.001 is highly significant.

Table [2]: Comparison between groups according to HAQ.

		Early Group[n=30]	Late Group[n=30]	Test value	p-value
HAQ	Mean±SD	1.83±0.52	1.15±0.33	5.163	<0.001**
	Range	0.25-2.5	0-1.875		
HAQ level	Mild disability	2[6.7%]	12[40.0%]	16.170	<0.001**
	Moderate disability	19[63.3%]	18[60.0%]		
	Severe disability	9[30.0%]	0[0.0%]		

Using: t-Independent Sample t-test for Mean±SD; x^2: Chi-square test for Number [%] or Fisher's exact test, when appropriate; **p-value <0.001 is highly significant

Table [3]: Comparison between groups according to Inflammatory markers.

	Inflammatory markers	Early Group[n=30]	Late Group[n=30]	Control Group[n=30]	Test value	p-value
Chemerin[ng/ml]	Mean±SD	389.21±112.79A	235.05±9.15B	169.86±27.52C	28.862	<0.001**
	Range	244.05-1094.6	227.92-277.61	115.36-218.38		
ESR[mm/h]	Median[IQR]	30[20-46.3]B	45[25.8-62.5]A	8[8-12]C	23.945	<0.001**
	Range	10-110	12-115	6-22		
CRP m[g/L]	Median[IQR]	10.5[4-17.3]A	12.8[8.3-42]A	2[0-3]B	7.225	<0.001**
	Range	1.7-190	1-110	0-3		

Using: One way Analysis of Variance test was performed for Mean±SD & Multiple comparison between groups through Post Hoc test: Tukey's test ; Kruskal–Wallis was performed for Median [IQR] & Multiple comparison between groups through Mann-Whitney test ; Different capital letters indicate significant difference at [p<0.05] among means in the same row; p-value >0.05 is insignificant; *p-value <0.05 is significant; **p-value <0.001 is highly significant.

Table [4]: Correlation between Chemerin, ESR and CRP m with different parameters in all study group, using Spearman's rank correlation coefficient.

	Chemerin[ng/ml]		ESR[mm/h]		CRP m[g/L]	
	r-value	p-value	r-value	p-value	r-value	p-value
Chemerin[ng/ml]			0.235	0.026*	0.278	0.008*
ESR[mm/h]	0.235	0.026*			0.670	<0.001**
CRP m[g/L]	0.278	0.008*	0.670	<0.001**		
Age[years]	0.088	0.410	0.214	0.043*	0.136	0.203
Height[m]	-0.100	0.348	-0.233	0.027*	-0.261	0.013*
Weight[kg]	0.096	0.367	0.014	0.893	-0.048	0.653
BMI [wt/[ht]^2]	0.144	0.176	0.157	0.139	0.127	0.234
Duration of disease[months]	-0.385	0.002*	0.330	0.010*	0.245	0.060
DAS CRP	0.395	0.002*	0.151	0.249	0.156	0.235
HAQ	0.451	<0.001**	0.087	0.510	0.099	0.452
RF[IU/ml]	0.236	0.025*	0.539	<0.001**	0.524	<0.001**
Anti CCP[u/ml	0.114	0.287	0.468	<0.001**	0.327	0.002*

Spearman's rank correlation coefficient [r]; p-value >0.05 is insignificant; *p-value <0.05 is significant; **p-value <0.001 is highly significant.

Table [5]: ROC curve for diagnostic performance between Early Group vs. Control Group according to serum level of Chemerin in rheumatoid arthritis patients.

Items	Cut-off	Sensitivity	Specify	PPV	NPV	AUC[C.I.95%]	p-value
Chemerin[ng/ml]	>218.38	100%	100%	100%	100%	1.00[0.94-1.00]	<0.001**
ESR[mm/h]	>19	83.3%	96.7%	96.2%	85.3%	0.95[0.86-0.99]	<0.001**
CRP m[g/L]	>3	80%	100%	100%	83.3%	0.91[0.81-0.97]	<0.001**

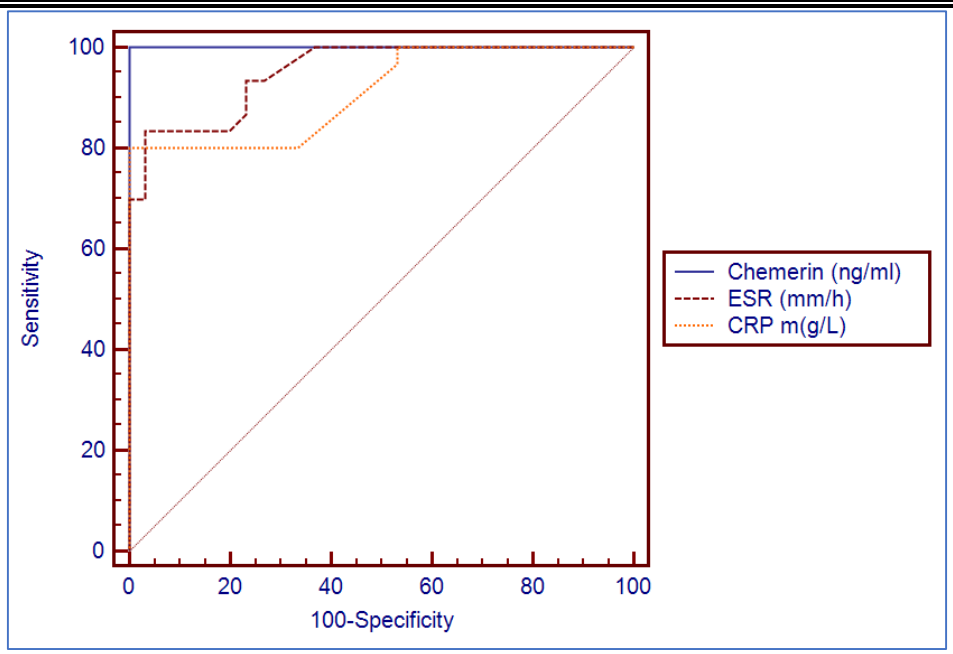

Figure [8]: Receiver-operating characteristic [ROC] curve for diagnostic performance between Early Group vs. Control Group according to serum level of Chemerin in rheumatoid arthritis patients.

DISCUSSION

This study aims to spotlight into finding out the relation between serum level of Chemerin and occurrence of rheumatoid arthritis disease. In addition to find out its relation to disease activity and severity assessed clinically and radiologically. The current study revealed significant correlations between Chemerin levels and key inflammatory markers, including ESR and CRP, suggesting a potential role for Chemerin as a valuable biomarker in the assessment of disease activity and severity in the studied autoimmune condition. Chemerin levels were found to have statistically significant positive correlations with ESR and CRP, all of which had p-values less than 0.05. These findings suggest that chemerin may have clinical relevance when used in conjunction with these inflammatory markers and measures of disease severity. In addition to this, the statistically significant positive correlation between Chemerin, CRP and ESR highlights the possibility that chemerin is related in expressing to the processes of inflammation associated to the disease. In agreement with our findings, **Mohammed Ali *et al.*** [15] reported that RA patients' chemerin levels are significantly higher than those of healthy controls.

Our study found a clear connection between the duration of disease and Chemerin level. the data demonstrates a significant negative relationship with P-value [P< 0.001]. There is a noticeable decrease in Chemerin levels in the patient population as the disease progresses. This significant finding indicates the possibility of a connection between Chemerin and the onset or progression of the disease being investigated. The negative relationship suggests that Chemerin levels tend to decrease with increasing rheumatoid arthritis duration, and this connect is significant. However, in **Gonzalez-Ponce *et al.*** [16], the found results showed that this variable was not different.

Although **Vazquez-Villegas *et al.*** [17] and **Tolusso *et al.*** [18] studies were unable to find any significant differences or variances in the Chemerin level and duration of disease that were examined. This could be because we split up the RA patients to early and late, even though that wasn't their intention at the time. According to previous research, our study sought to address potential gender-related biases by including both male and female participants. The analysis revealed a noteworthy and statistically significant difference among the groups based on sex. Notably, the control group exhibited a higher frequency of male participants compared to both the early and late groups. This deliberate inclusion of males in our study design providing a more comprehensive understanding of the observed effects. This approach stands in contrast to **Gonzalez-Ponce *et al.*** [16] exclusive focus on female participants only.

Our study shows the correlation observed between elevated Chemerin levels and increased disease activity in rheumatoid arthritis patients, it's noteworthy that heightened Chemerin levels often coincide with higher DAS 28 CRP measurements. This connection suggests a potential relationship between Chemerin and the severity of the disease. The concomitant elevation of both Chemerin and DAS 28 CRP underscores a possible interplay between these factors in influencing the progression or severity of rheumatoid arthritis. which agreement with previous studies which investigated relation between Chemerin and disease activity in rheumatoid patients. **Gonzalez-Ponce *et al.*** [16] investigated 210 patients with RA and found that Chemerin levels were correlated with DAS28 and that these levels were higher in active RA. **Vazquez-Villegas *et al.*** [17] evaluated 82 patients with RA which divided to 43 RA patient with functional disability and 39 RA patient without disability and observed positive correlation between Chemerin and DAS. However, they used DAS 28 ESR. Further exploration of this relationship could offer deeper insights into the multifaceted nature of the disease's manifestations and potentially aid in refining therapeutic approaches.

Moreover, our study revealed a notable association between elevated Chemerin levels and higher scores in the Health Assessment Questionnaire [HAQ], indicative of increased disease disability. This finding suggests a potential utility for Chemerin as a predictive tool for assessing disease disability in the future. The observed correlation between high Chemerin levels and heightened HAQ scores underscores the clinical relevance of Chemerin as a biomarker, while proposing the prospect of incorporating Chemerin assessments into prognostic frameworks for a more comprehensive understanding of rheumatoid arthritis disability. These findings have been supported by other groups: **Vazquez-Villegas *et al.*** [17] observed 82 patients with RA which divided to 43 RA patient with functional disability and 39 RA patient without disability and authors identified a correlation between Chemerin and HAQ-Di scores.

Our findings indicate that rheumatoid drugs do not impact Chemerin levels; instead, these levels are solely influenced by the disease. However, **Vazquez-Villegas *et al.*** [17] found positive correlation between Chemerin and MTX, in spite of other drugs showed no significant difference. In addition to we added JAKi in our study which has no significant difference. This highlights the diagnostic potential of Chemerin, serving

as a consistent marker for monitoring disease progression regardless of drug usage. This stability of Chemerin levels, unaffected by rheumatoid drugs, enhances its value in diagnosing and tracking the development of rheumatoid arthritis. It offers clinicians a reliable and disease-specific indicator, ensuring effective management strategies for patients.

The relatively small number of participants is the primary limitation of our investigation. While the information gathered from our participants was useful, the small sample size may limit the generalizability of our findings to a larger population. Future research projects could benefit from larger and more diverse participant groups in order to improve the robustness and external validity of the results. Through an in-depth examination of the accuracy of diagnosis in distinguishing between those with Early and Late RA groups in relation to a Control group using serum levels of Chemerin, CRP and ESR. For every single biomarker, the most appropriate cut-off values have been identified using the Receiver Operating Characteristic [ROC] curves, which made it possible to evaluate each biomarker's sensitivity, specificity, PPV, NPV, and area under the curve [AUC].

Having a cut-off value of >218.38 ng/ml, Chemerin demonstrated remarkable diagnostic performance among the Early RA group. This resulted in 100% sensitivity, specificity, PPV, and NPV as well as an AUC of 1.00 [95% CI: 0.94-1.00], indicating ideal discriminatory abilities. By comparison, the AUC values of 0.95 and 0.91 for ESR [>19 mm/h] and CRP [>3 g/L] indicated significant diagnostic accuracy as well. For each of the three biomarkers, the p-values [p<0.001] show a substantial difference in discrimination between the Early RA group and the Control group. Chemerin demonstrated remarkable ability to differentiate for the Late RA group as well, with an AUC of 1.00 [95% CI: 0.94-1.00] and excellent sensitivity, specificity, PPV, and NPV while maintaining a cut-off of >218.38 ng/ml. Strong diagnostic accuracy was maintained by CRP and ESR, with AUC values of 0.95 and 0.99, respectively. Chemerin continuously showed better discrimination capacity while comparing the diagnostic performance between the Early and Late RA groups, as seen by its perfect AUC of 1.00 in both scenarios. High discriminatory ability was also shown by ESR and CRP, with slightly greater AUC values in the Late RA group. The results highlight the potential value of serum Chemerin levels over established indicators ESR and CRP as an effective biomarker for differentiating between Early and Late RA as well as among those who have rheumatoid arthritis and disease-free individuals. With this knowledge, practitioners will have a more accurate and dependable tool for patient stratification, which could have significant consequences for the early identification and treatment of RA. Nevertheless, moving from research discoveries to practical clinical applications requires thorough validation in more extensive and varied patient populations.

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