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Study of Serum Monocyte Chemoattractant Protein-1AS A Marker in Rheumatoid Arthritis

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic, multisystem autoimmune disease which manifests itself in multiple joints of the body. It is characterized by infiltration of inflammatory cells such as monocytes and it is believed to be the result of a faulty immune response.

Chemokines play a major role in selectively recruiting monocytes, neutrophils, and lymphocytes, as well as in inducing chemotaxis through the activation of G-protein-coupled receptors. Monocyte chemoattractant protein-1 (MCP-1/CCL2) is one of the key chemokines that regulates migration and infiltration of monocytes/macrophages. **Aim of The Work:** was to study the role of serum Monocyte chemotactic protein-1 (MCP-1) in patients with rheumatoid arthritis as a diagnostic and prognostic marker and the possible association with disease activity. **Subjects and methods**: Forty rheumatoid arthritis diseased patients were selected , they were 3 males and 37 females. The patients were categorized into two groups according to activity of the disease regarding DAS score . Another 20 healthy subjects, 2 males and 18 females, with no history of rheumatoid disease were recruited as controls .Results: this study showeda highly significant increase in MCP-1 and ESR in all rheumatoid arthritis patients groups , active rheumatoid arthritis patients group and inactive rheumatoid arthritis patients group compared to control group. Correlation study of serum MCP-1 revealed a significant positive correlation between serum MCP-1 and ESR and DAS score in all patients versus the healthy group and a significant positive correlation between serum MCP-1 and ESR in the active group. ROC curve analysis was showing the diagnostic performance of serum MCP-1 in rheumatoid arthritis patients(active and inactive) versus the healthy control group, at a cut-off level of 52.5 ng/ml., the diagnostic sensitivity, specificity, negative predictive value and positive predictive value were 98%, 93%, 93% and 98% respectively. Also, it shows the diagnostic performance of serum MCP-1 in discriminating active rheumatoid arthritis patients from the inactive group, at a cut-off level of 61ng/ml **Conclusion:** - Serum MCP-1 is one of the best indicator of clinical arthritic activity in RA patients. It represents a novel, independent indicator of clinical arthritic activity that also provides a good reflection of effect of treatment in rheumatoid arthritis patients.

Key words: Rheumatoid arthritis (RA), serum Monocyte chemotactic protein-1 (MCP-1)

INTRODUCTION

Rheumatoid arthritis (**RA**) is an autoimmune disease that results in a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks flexible (synovial) jointsThe inflammatory process is characterized by infiltration of inflammatory cells into the joints, leading to proliferation of synoviocytes and destruction of cartilage and bone. In RA synovial tissue, the infiltrating cells such as macrophages, T cells, B cells and dendritic cells play important role in the pathogenesis of RA. Migration of leukocytes into the synovium is a regulated multi-step process, involving interactions between leukocytes and endothelial cells, cellular adhesion molecules. [1]Many data suggest that the disease involves abnormal B cell–T cell interaction, once the abnormal immune response has become established (which may take several years before any symptoms occur), plasma cells derived from B lymphocytes produce rheumatoid factors and Anti-citrullinated protein antibodies (ACPA) of the IgG and IgM classes in large quantities. They appear to activate macrophages through Fraction crystalline (Fc) receptor and perhaps complement binding. This can contribute to inflammation of the synovium. Synovial macrophages and dendritic cells further function as antigen presenting cells by expressing Major Histo-Compitability, leading to an established local immune reaction in the tissue [2].

Numerous cytokines are expressed and are functionally active in the synovial tissue once the disease has developed [3].Nonetheless, it is well recognized that ongoing inflammation of the peripheral joints with accompanying tissue

damage involves complex, cytokine-driven interactions between resident synovial and infiltrating inflammatory cells [4],particularly T-helper 1 (Th1) cells of the effector-memory phenotype [5]. Many studies have suggested that chronic inflammation in the rheumatoid joint may result from the sustained activation/dysregulation of inflammatory cytokine networks which operate independently of triggering autoantigens and T-cell receptor (TCR) ligation [6]. In this setting, two mechanisms, probably interactive, have been identified which appear to maintain autoantigen-independent production of inflammatory cytokines. These are (i) direct activation of a subset of effector-memory Th1 cells by cytokines signaling via the interleukin-2 receptor (IL-2R) common γ-chain in combination with IL-12 and IL-18, with resultant generation of interferon-γ (IFN-γ) [7],and (ii) continuous activation of immune and inflammatory cells, including Th1 cells, via interaction of Toll-like receptors (TLRs) with extracellular matrix components released from damaged host tissues [8].

Chemokines, a superfamily of small (8–14-kd), structurally related chemotactic cytokines, have been reported to selectively recruit and activate leukocytes at sites of inflammation. These chemokines can be divided into 2 major subfamilies, the CXC and CC chemokines. CXC chemokines such as interleukin-8 (IL-8) have been implicated in acute inflammation, since they exert their function mainly on neutrophils, whereas the CC chemokines, including RANTES and monocyte chemoattractant protein 1 (MCP-1), attract and activate a variety of cells, including monocytes, macrophages, lymphocytes, eosinophils, and basophils, and have been implicated in chronic inflammatory disease [9].

The monocyte chemoattractant protein-1 (MCP-1/CCL2) is a potent chemotactic factor for monocytes [10] . CCL2 is the first discovered human CC chemokine. Located on chromosome 17 (chr.17, q11.2), human MCP-1 is composed of 76 amino acids and is 13 kDa in size. MCP belongs to a family composed of at least four members (MCP-1, −2, −3, and −4) [11].

CCL2 is produced by various cells, either constitutively or after induction by oxidative stress, cytokines, or growth factors [12]. As MCPs have a broad cell spectrum, their receptors are expressed on various leukocyte types. In addition, all human MCPs are known to bind to at least two receptors. Also, it should be noted that many receptors such as C-C chemokine receptor type 2 (CCR2) respond to several different ligands13 It is important to note that CCR2 has dual roles and has both pro-inflammatory and anti-inflammatory actions. 9CCL2 has been demonstrated to recruit monocytes into foci of active inflammation. Apart from recruiting and directing leukocyte movement, several lines of evidence indicate that CCL2 might influence T-cell immunity 14

SUBJECT AND METHODS

This study was conducted on 40 adult patients with rheumatoid arthritis disease from Rheumatology outpatient clinic at El Zahra University Hospital in the period from December 2012 to April 2013.They were 3 males and 37 females. The patients were categorized according to activity of the disease regarding DAS score into two groups .

Group 1: Active rheumatoid arthritis with DAS28 >2.6.

Group 2: Inactive rheumatoid arthritis with DAS28 <2.6.

Another 20 apparently healthy subjects, 2 males and 18 females, with no history of rheumatoid disease were selected as a control group.

For subjects of all groups the consent was taken and history was taken. All individuals in this study were subjected to the following:

•Full history was taking.

•Complete clinical examination.

3)Blood samples were collected from all subjects .The collected blood was divided among an EDTA tube for complete blood picture, sodium citrate tube for ESR, fluoride oxalate tube for sugar and a plain tube for serum separation . A fresh serum aliquots from each individual was used for assay of blood suger, serum creatinine and C-reactive protein)CRP).The other aliquot was stored at -20C until the assay of monocyte chemoattractante protein-1 . Laboratory investigations include:

a) CBC was done on coulter counter T660 (Coulter Electronics, Hielaeh, FL.(USA).

b)Erythrocyte Sedimentation **Rate (ESR):**

ESR was measured by Westerngrenmethod[9]

c) Random Blood Sugar:

By Roche / Hitachi 912 **(Roche Dignostic , Indianapolis, IN USA).**In El Zahraa University Hospital.

d) Routine Kidney Function Tests: Routine Kidney function Tests were measured on Roche / Hitachi 912 **(Roche Diagnostic , Indianapolis, IN USA)**. In Al Zahraa University Hospital.

e) C-reactive protein.
The CRP Direct Latex reagent[8].
f)Rheumatiod factor. The RF Direct Latex reagent **[8].**
g)Serum Creatinine level.was measured by a modified rate Jaffe' method [3].
Serum MCP-1 and assayed by ELISA technique using reagents provided by Glory Science Co., Ltd (2400 Veterans Blvd. (Suite 16 - 101,Del Rio, TX 78840, USA)according to [17] .
Data was analyzed by Microsoft Office 2003 (excel) and Statistical Package for Social Science (SPSS) version 16.

RESULTS

Results of the present study are shown in Tables 1-6 and Figures 1-5 .The study showed that :
Statistical comparison between the various studied parameters in rheumatoid arthritis patients collectively and the healthy control group revealed a highly significant increase in MCP-1 and ESR in rheumatoid arthritis patients ($p<0.01$; $p<0.01$; respectively).with a non- significant difference in age, s.Creatinine, RBCs, PLT, MCV, MCH, MCHC, and s.Glucose($p>0.05$). In addition there was a significant increase in s.Urea and WBCs in rheumatoid arthritis patients ($P<0.05$; $P<0.05$; $P<0.05$; $P<0.05$ respectively).And also there was highly significant decrease in Hb in rheumatoid arthritis patients ($p<0.01$).Table 1.Statistical comparison between the various studied parameters in active rheumatoid arthritis patients and the healthy control group revealed a highly significant increase in MCP-1 and ESR in active rheumatoid arthritis patients ($p<0.01$; $p<0.01$; respectively).with a non- significant difference in age, s.Creatinine, WBCs, RBCs, PLT, MCV, MCH, MCHC and s.Glucose . In addition there was a significant increase in s.Urea in active rheumatoid arthritis patients($P<0.05$; $P<0.05$ respectively).And also there was highly significant decrease in Hb in active rheumatoid arthritis patients ($p<0.01$)as shown in Table 2.
Statistical comparison between the various studied parameters in inactive rheumatoid arthritis patients and the healthy control group revealed a highly significant increase in MCP-1 and ESR in inactive rheumatoid arthritis patients ($p<0.01$; $p<0.01$; respectively).with a non -significant difference in age, s.Creatinine, WBCs, RBCs, PLT, MCV, MCH, MCHC, and s.Glucose. In addition there was a significant increase in s.Urea in inactive rheumatoid arthritis patients compared to control group ($P<0.05$) . And also there was highly significant decrease in Hb in inactive rheumatoid arthritis patients($p<0.01$). Table.3.
Statistical comparison between the various studied parameters in active rheumatoid arthritis patients as compared to the inactive group revealed a highly significant increase in ESR in active rheumatoid arthritis patients ($p<0.01$). In addition there was a significant increase in MCP-1 in active rheumatoid arthritis patients ($P<.05$). with a non-significant difference in all other parameters Table.4

Table1: Statistical comparison between the various studied parameters in all rheumatoid arthritis patients as compared to control group.

Group / Parameter	Control Group(n=20) Mean ±SD	All Patients(n=40) Mean ±SD	P
Age(years)	**40.33 ± 7.46**	**43.25 ± 8.06**	>0.05
s.creatinine (mg/ml)	**0.60 ± 0.13**	**0.71 ± 0.22**	>0.05
s. urea (mg/ml)	**21.66 ± 4.98**	**27.5 ± 7.64**	<0.05
WBCs (x10/cmm)	**7.25 ±1.49**	**7.37 ± 2.82**	<0.05
RBCs (x10/cmm)	**4.62 ± 0.33**	**4.60 ± 1.04**	>0.05
Hb (g/dl)	**12.63 ± 0.52**	**11.85 ± 1.09**	<0.01
PLT (/cmm)	**291.73 ± 55.72**	**292 ± 93.29**	>0.05
MCV (fL)	**81.4 ± 4.20**	**80.96 ± 6.07**	>0.05
MCH (Pg)	**27.72 ± 1.72**	**27.12 ± 2.34**	>0.05
MCHC (g/dL)	**33.94 ± 0.82**	**33.67 ± 1.18**	>0.05
s.Glucose (mg/ml)	**83.26 ± 12.19**	**94.97 ± 33.74**	>0.05
MCP-1 (ng/dl)	**39.92 ± 8.69**	**90.16 ± 49.61**	<0.01
ESR (mm/h)	**6.2 ± 3.43**	**47.62 ± 17.46**	<0.01

P>0.05: Non-significant.P<0.05: Significant. p<0.01, <0.001: Highly significant.

Table2: Statistical comparison between the various studied parameters in active rheumatoid arthritis patients as compared to control group.

Group / Parameter	Control Group (n=20) Mean ±SD	Active Group (n=20) Mean ±SD	P
Age(years)	**40.33 ± 7.46**	**44.45 ± 7.88**	>0.05
s.creatinine (mg/ml)	**0.60 ± 0.13**	**0.69 ± 0.19**	>0.05
s. urea (mg/ml)	**21.66 ± 4.98**	**29.35 ± 8.57**	<0.05
WBCs (x10/cmm)	**7.253 ±1.49**	**7.61 ± 3.46**	>0.05
RBCs (x10/cmm)	**4.62 ± 0.33**	**4.35 ± 0.54**	>0.05
Hb (g/dl)	**12.63 ± 0.52**	**11.87 ± 1.19**	<0.05
MCV (fL)	**81.4 ± 4.20**	**80.73 ± 5.78**	>0.05
MCH (Pg)	**27.72 ± 1.72**	**27.24 ± 2.26**	>0.05
MCHC (g/dL)	**33.94 ± 0.82**	**33.73 ± 1.19**	>0.05
s.Glucose (mg/ml)	**83.26 ± 12.19**	**101.4 ± 46.06**	>0.05
MCP-1 (ng/dl)	**39.92 ± 8.69**	**108.14 ± 65.17**	<0.01
ESR (mm/h)	**6.2 ± 3.43**	**59 ± 15.02**	<0.01

P>0.05: Non-significant.
P<0.05: Significant.
p<0.01, <0.001: Highly significant.

Table3: Statistical comparison between the various studied parameters in inactive rheumatoid arthritis patients as compared to control group.

Group / Parameter	Control Group (n=20) Mean ±SD	Inactive Group (n=20) Mean ±SD	P
Age(years)	**40.33 ± 7.46**	**40.05 ± 7.84**	>0.05
s.creatinine (mg/ml)	**0.60 ± 0.13**	**0.72 ± 0.25**	>0.05
s. urea (mg/ml)	**21.66 ± 4.98**	**25.65 ± 6.26**	<0.05
WBCs (x10/cmm)	**7.25 ±1.49**	**7.13 ± 2.06**	>0.05
RBCs (x10/cmm)	**4.62 ± 0.33**	**4.85 ± 1.33**	>0.05
Hb (g/dl)	**12.63 ± 0.52**	**11.82 ± 1.00**	<0.05
MCV (fL)	**81.4 ± 4.20**	**81.19 ± 6.48**	>0.05
MCH (Pg)	**27.72 ± 1.72**	**27 ± 2.46**	>0.05
MCHC (g/dL)	**33.94 ± 0.82**	**33.61 ± 1.21**	>0.05
s.Glucose (mg/ml)	**83.26 ± 12.19**	**88.55 ± 11.36**	>0.05
MCP-1 (ng/dl)	**39.92 ± 8.69**	**72.19 ±11.14**	<0.01
ESR (mm/h)	**6.2 ± 3.43**	**35.75 ± 12.53**	<0.01

P>0.05: Non-significant.
P<0.05: Significant.
p<0.01, <0.001 Highly significant

Table4: Statistical comparison between active compared to inactive patient .

Group Parameter	Active Group (n=20) Mean ±SD	Inactive Group (n=20) Mean ±SD	P
Age(years)	**44.45 ± 7.88**	**40.05 ± 7.84**	>0.05
s.creatinine (mg/ml)	**0.69 ± 0.19**	**0.72 ± 0.25**	>0.05
s. urea (mg/ml)	**29.35 ± 8.57**	**25.65 ± 6.26**	>0.05
WBCs (x10/cmm)	**7.61 ± 3.46**	**7.13 ± 2.06**	>0.05
RBCs (x10/cmm)	**4.35 ± 0.54**	**4.85 ± 1.33**	>0.05
Hb (g/dl)	**11.87 ± 1.19**	**11.82 ± 1.00**	>0.05
MCV (fL)	**80.73 ± 5.78**	**81.19 ± 6.48**	>0.05
MCH (Pg)	**27.24 ± 2.26**	**27 ± 2.46**	>0.05
MCHC (g/dL)	**33.73 ± 1.19**	**33.61 ± 1.21**	>0.05
s.Glucose (mg/ml)	**101.4 ± 46.06**	**88.55 ± 11.36**	>0.05
s.AST (mg/dL)	**30.05 ± 22.38**	**25.1 ± 8.13**	>0.05
s.ALT (mg/dL)	**30.2 ± 16.87**	**29 ± 9.11**	>0.05
MCP-1 (ng/dl)	**108.14 ± 65.17**	**72.19 ±11.14**	<0.05
ESR (mm/h)	**59 ± 15.02**	**35.75 ± 12.53**	<0.01

P>0.05: Non-significant .
P<0.05: Significant .
p<0.01, <0.001: Highly significant .

The correlation study between serum MCP-1 and other studied parameters of all rheumatoid arthritis patients is shown in Table 4 . It revealed a significant positive correlation between serum MCP-1 and ESR and DAS score (MCP-1=.0.478, 0.468respectivly) (P<0.05).Table.5 & Figs.1,2

Table5: Correlation study between Serum MCP-1 and Other Studied Parameters in all rheumatoid arthritis patients.

Patients	MCP-1	P value
Age	0.265	> 0.05
Creatinine (mg/dL)	0.059	> 0.05
WBCs ($X10^3$/cmm)	0.069	> 0.05
RBCs (M/cmm)	0.003	> 0.05
HB(g/dL)	-0.03	> 0.05
PLT(/cmm)	-0.224	> 0.05
MCV(fL)	-0.085	> 0.05
MCH(Pg)	-0.129	> 0.05
MCHC(g/dL)	-0.298	> 0.05
Urea(mg/dL)	0.097	> 0.05
Glucose(mg/dL)	-0.154	> 0.05
ESR(mm/h)	0.478	< 0.01
DAS score	0.468	< 0.01

P>0.05: Non-significant correlation.
P<0.05: Significant correlation.

Figure 1: Correlation between s.MCP-1(ng/mL) and ESR(mm/h) in all rheumatoid patients.

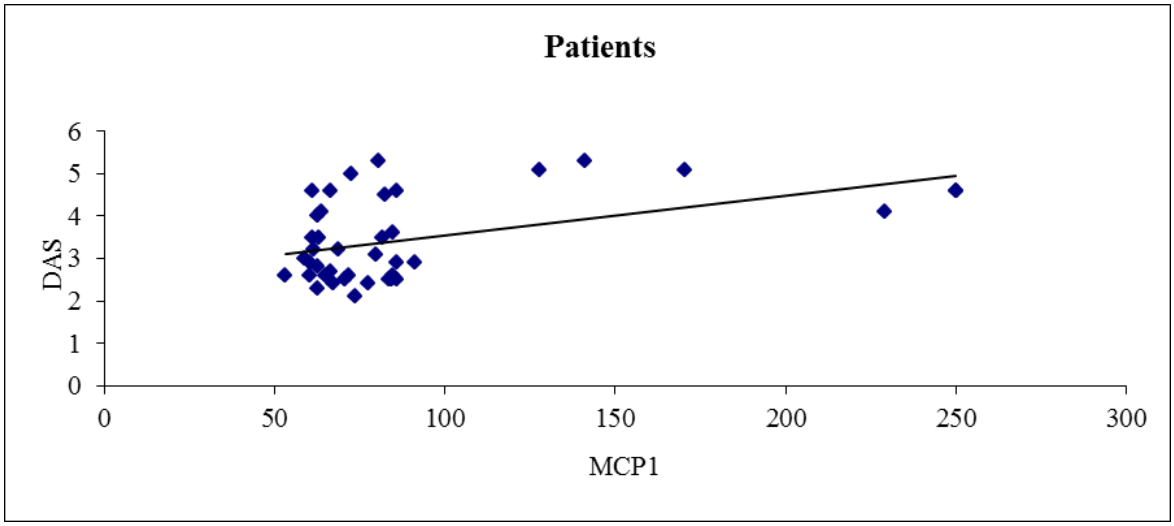
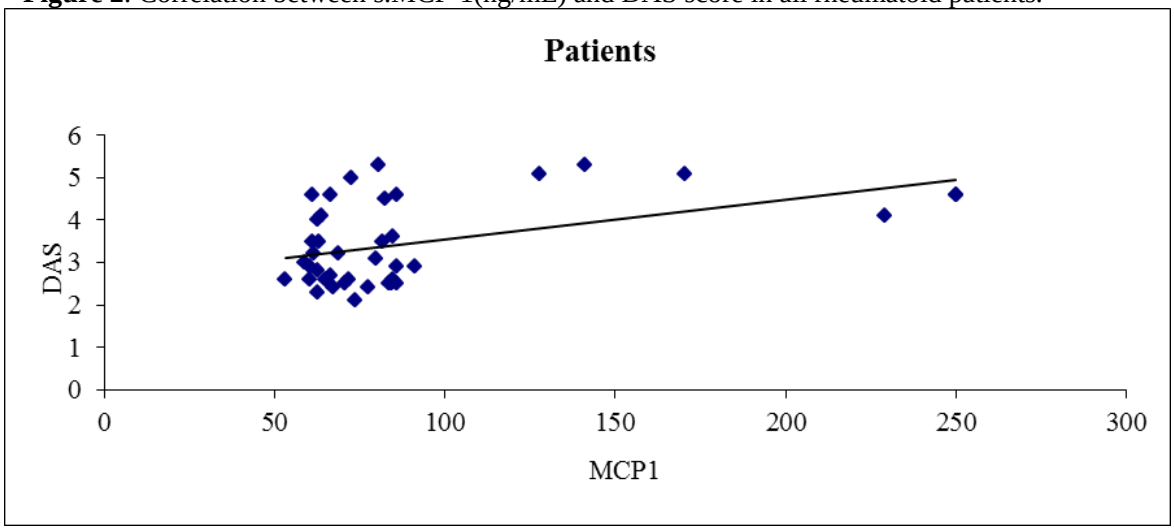


Figure 2: Correlation between s.MCP-1(ng/mL) and DAS score in all rheumatoid patients.



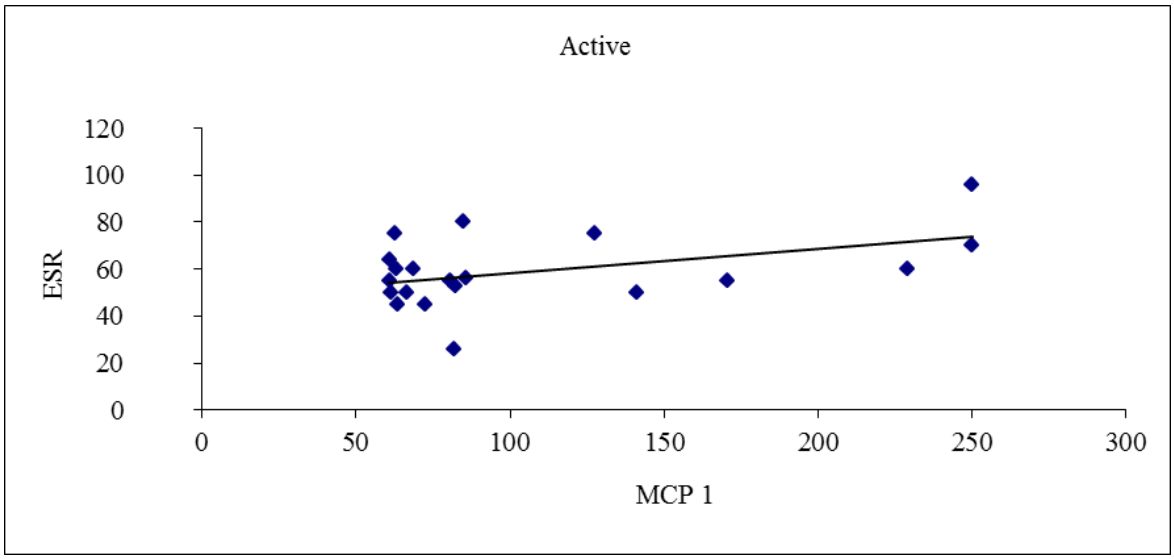
The correlation study between serum MCP-1 and other studied parameters in active rheumatoid arthritis patients is shown in Table 5 . It revealed a significant positive correlation between serum MCP-1 and ESR (ESR =0.456)($P<0.05$).Tab.6 &Fig.3

Table 6: Correlation study between Serum MCP-1 and Other Studied Parameters in active rheumatoid arthritis patients.

Active	MCP-1	P value
Age	0.188	> 0.05
Creatinine (mg/dL)	0.214	> 0.05
WBCs (X10³/cmm)	0.043	> 0.05
RBCs (M/cmm)	0.192	> 0.05
HB(g/dL)	-0.076	> 0.05
MCV(fL)	-0.141	> 0.05
MCH(Pg)	-0.303	> 0.05
MCHC(g/dL)	-0.442	> 0.05
Urea(mg/dL)	0.04	> 0.05
Gluocse(mg/dL)	-0.248	> 0.05
ESR(mm/h)	0.456	< 0.05
DAS score	0.344	> 0.05

P>0.05: Non-significant correlation.
P<0.05: Significant correlation.

Figure 3: Correlation between s.MCP-1(ng/mL) and ESR(mm/h) in active rheumatoid arthritis patients.



The correlation study between serum MCP-1 and other studied parameters in inactive rheumatoid arthritis patients is shown in Table 7. It revealed a non-significant correlation between serum MCP-1 and other studied parameters.

Table 7: Correlation study between Serum MCP-1 and Other Studied Parameters in inactive rheumatoid arthritis patients.

Inactive	MCP-1	P value
Age	0.103	> 0.05
Creatinine (mg/dL)	-0.295	> 0.05
WBCs (X10³/cmm)	0.049	> 0.05
RBCs (M/cmm)	0.205	> 0.05
HB(g/dL)	0.148	> 0.05
MCV(fL)	0.126	> 0.05
MCH(Pg)	0.34	> 0.05
MCHC(g/dL)	-0.287	> 0.05
Urea(mg/dL)	-0.236	> 0.05
Gluocse(mg/dL)	-0.235	> 0.05
AST(U/L)	-0.173	> 0.05
ALT(U/L)	-0.29	> 0.05
ESR(mm/h)	-0.208	> 0.05
DAS score	0.011	> 0.05

P>0.05: Non-significant correlation.P<0.05: Significant correlation.

Receiver –operating characteristics (ROC) curve analysis was applied to assess the diagnostic performance of serum MCP-1 in rheumatoid arthritis patients versus the healthy control group. At a cut-off level of 52.5 ng/ml, the diagnostic sensitivity, specificity, negative predictive value and positive predictive value were 98%, 93%, 93% and 98% respectively.Fig.4

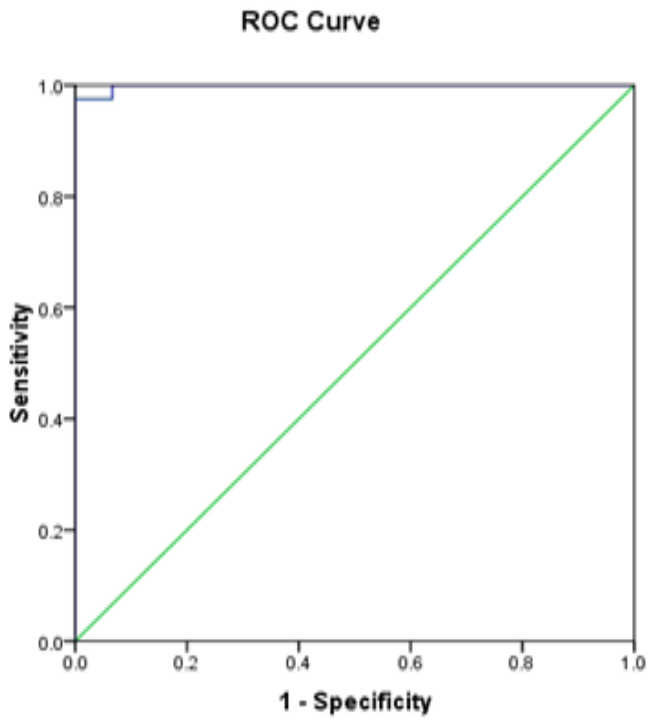


Figure 4: ROC-curve analysis showing the diagnostic performance of serum MCP-1 in rheumatoid arthritis patients versus the healthy control group

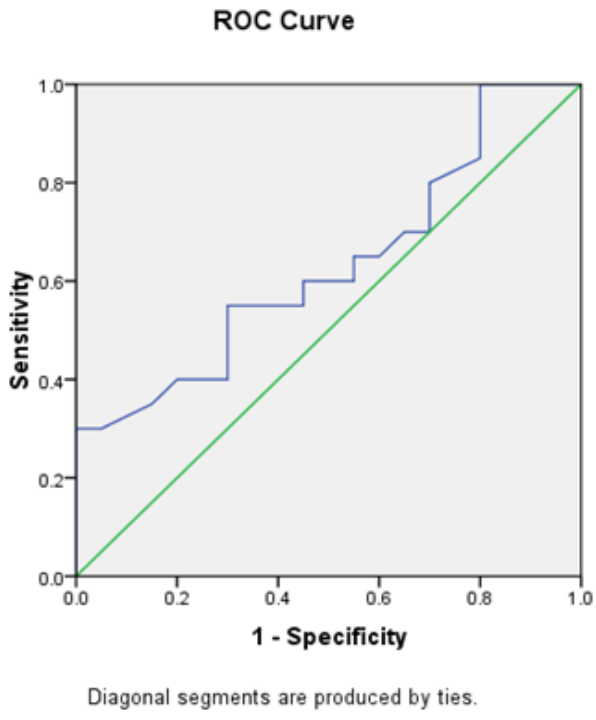


Figure 5: ROC- curve analysis was applied to assess the diagnostic performance of serum MCP-1 in discriminating active rheumatoid arthritis patients fromthe inactive group. At a cut-off level of 61ng/ml.

DISCUSSION

Rheumatoid arthritis (RA) is a chronic systemic disorder characterized by the development of new capillaries that are involved in the infiltration of inflammatory cells which results in synovial hyperplasia and progressive destruction of cartilage and bone. Synovial tissue (ST) lining consists of macrophages and fibroblasts that have profound effects in the destructive process in RA, via production of proinflammatory cytokines, chemokines, and proangiogenic factors**.**[18]

A complex network of adhesion molecules and chemokines co-ordinate cell migration, by working in concert to induce an inflammatory response **.**[19] Monocyte chemotactic protein-1 (MCP-1), is chemotactic for monocytes, basophils, T cells, and mast cells . Moreover, an MCP-1 antagonist prevented or reduced arthritis in MRL-lpr mice[20]

DAS28 (with inclusion of ESR) has long been used in daily clinical practice to monitor disease activity in RA patients . Therefore, it remains to be resolved whether MCP-1 can supplant CRP and ESR in monitoring RA disease activity by use of the DAS 28 formula and its components[21]

The reported associations in RA patients between blood MCP-1 level and swollen joint count (SJC), and between the plasma MCP-1 level and erythrocyte sedimentation rate (ESR) and CRP level, are not entirely consistent . This discrepancy has not been resolved. In addition, the manner in which blood MCP-1 level relates to measures of clinical arthritis in RA patients (especially disease activity score 28: DAS28) remains unknown[22]**.**

Serum CRP and blood ESR levels have long been routinely used to monitor clinical arthritic activity in RA patients for many years . It is uncertain whether other biomarkers are useful in monitoring RA patients[7].

We found that plasma MCP-1 particularly, the adapted DAS28-MCP-1 was useful in evaluating RA disease activity. Furthermore, there was positive correlation between MCP-1 levels and ESR .

We hypothesized that RA clinical disease activity is more accurately reflected by locally produced MCP-1 than by CRP and ESR levels (whether produced systemically or outside the arthritic joint). To test this hypothesis, we estimated correlations between blood MCP-1, CRP, and ESR, visual analog scale for disease activity score 28 (DAS28).

Based on the previous observations, the aim of the present work was to assess serum levels of monocyte chemotactic protein-1 in a group of patients with different stages of rheumatoid disease activity in order to evaluate its clinical utility in assessment of disease activity and thus allowing the initiation of preventive therapeutic measures in a timely manner.

This study revealed that serum MCP-1 and ESR were highly significant increase in rheumatoid arthritis patients when compared with those of the healthy control subjects ($p<0.01$, $p<0.01$;respegtively) (Table 1).

This is in accordance with the study done by **Liou et al. (2013)**[18]theyrecorded a significant increase in serum MCP-1 and ESR levels in rheumatoid arthritis patients than control group and attributed these finding to the fact that rheumatoid arthritis patients frequently have inflamed synovial joints.

The present study revealed that, there were highly significant increase in serum MCP-1 and ESR levels in active rheumatoid arthritis patients as compared with controls ($p<0.01$, $p<0.01$;respegtively) .

Also, in a study performed by**Liou et al. (2013)**[18] ,on 111 patients with RA serum MCP-1 and ESR were highly significant increase in active rheumatoid arthritis patients as compared with controls and DAS28-MCP-1 were designated as measures of clinical arthritic activity.

Serum MCP-1 and ESR in the present study showed high significant elevation in inactive rheumatoid arthritis when compared with that of the control group ($p<0.01$, $p<0.01$;respegtively) .

In the present study, there was a highly significant increase in ESR in active rheumatoid arthritis patients compared to inactive rheumatoid patients ($p<0.01$). In addition there was a significant increase in MCP-1 in active rheumatoid arthritis patients than the inactive rheumatoid patients ($p<0.05$)

The study done by **Xi Bao (2010)**[23]**,**found that the expression of MCP-1 in simple RA patients was significantly higher than that in the control group. Also **Liou et al. (2013)**[18]**,** reportedthat high significant increase in MCP-1 and blood ESR in active rheumatoid arthritis patients was found when compared to the inactive group. These results suggest that the adapted MCP-1 is a useful indicator of clinical disease activity in RA.

As regard correlation study of the serum MCP-1 in rheumatoid arthritis patients revealed a significant positive correlation between serum MCP-1 and ESR- DAS score($p<0.01$, $p<0.01$;respegtively). These results are in agreement with**Liou et al. (2013)[18],**

The correlation results in the present study, between serum MCP-1 and other studied parameters in active rheumatoid arthritis patients revealed significant positive correlation between serum MCP-1 and ESR($p<0.05$) **Liou et al. (2013)[18],** They reported that among active rheumatoid group there was significant positive correlation between serum MCP-1 and ESR.

The correlation results in the present study, between serum MCP-1 and other studied parameters in inactive rheumatoid arthritis patients revealed a non-significant correlation($p >0.05$) .

Other results in this study are in agreement with **et al. (2013)[18].**Theyreported that serum MCP-1 was not significantly correlated with other studied parameters .

Serum urea was significantly higher in rheumatoid arthritis patients when compared to the healthy control group ($p<0.01$) .This is in agreement with the finding of **Gray and David (2002)[24],**who reported that higher levels of serum urea indicate a falling of GFR as a result of decreased capability of the kidney to excrete waste products as a result of usage of Nonsteroidal Antiinflammatory Drugs (NSAIDs) which become standard care in inflammatory conditions such as rheumatoid arthritis disease.

A study done by **van Wietmarschen et al** .(2012) **[25],**on 39 RA patients with different stages of disease activity showed significant increases of serum urea in active RA patients as compared to the control group and contributed these finding to the usage of 8017%%8017 **(NSAIDs**) that affect kidney functions.

In the present study there was a significant increase of serum urea in active RA patients as compared to the control group ($p<0.05$) .

However **Gu et al. (2012)[14], revealed** decreased levels of urea in active RA compared to inactive RA.

This study reported that serum urea, was significantly increase in inactive rheumatoid arthritis patients when compared with those of healthy control subjects ($p<0.05$) . This is in accordance with the study done by **[10],**who recorded significant increase in serum urea in inactive RA patients than control group and contributed these finding to the usage of NSAIDs which affect kidney functions.

In this study, there was highly significant decrease in HB in rheumatoid arthritis patients when compared to the healthy control groups ($p<0.01$) . This result is in agreement with **Adam andMuller (2005) [26],**who showed that anemia of chronic disease such as rheumatoid arthritis is associated with decrease HB level. The pathogenesis of the anemia of chronic disease is incompletely understood. Two major factors appear to be important: trapping of iron in macrophages, making it relatively unavailable for new hemoglobin synthesis; and inability of the morphologically normal marrow to increase erythropoiesis in response to the anemia[27].

Inflammatory mediators, particularly tumor necrosis factor-alpha (TNF-alpha), interleukin-1, interleukin-6, interleukin-10, and interferon gamma, contribute to these change .Hepcidin, an acute phase reactant produced by the liver, may play a key role in cytokine-mediated anemia, as this protein decreases intestinal iron absorption and iron release from macrophages[28].

In the present study Hb level was significantly decreased in active RA patients as compared to the healthy control group ($p<0.05$) . This is in agreement with **Adam andMuller (2005)**[26] they reported that decreased Hb level in anemia of inflammatory diseases such as RA as inflammatory diseases interfere with the body's ability to use stored iron and absorb iron from the diet. Anemia of inflammatory disease is easily confused with iron-deficiency anemia because in both forms of anemia, levels of iron circulating in the blood are low. Circulating iron is necessary for RBC production. Low blood iron levels occur in iron-deficiency anemia because levels of iron stored in the body's tissues are depleted. In Anemia of inflammatory disease, however, iron stores are normal or high. Low blood levels occur, despite normal iron stores, because inflammatory diseases interfere with the body's ability to use stored iron and absorb iron from the diet[26].

Also there was a significant decrease in Hb level in inactive rheumatoid arthritis when compared with those of healthy control subjects ($p<0.05$) (Table 3) . **Adam andMuller (2005) 26** showed that anemia of chronic disease such as rheumatoid arthritis is associated with decrease HB level.

The present study revealed significant increase in WBCs, these result are also with agreement with

Braunwald et al. (2012) [29] .who recorded a high white blood cell count which may be resulted from inflammation due to rheumatoid arthritis (RA).

Receiver –operating characteristics (ROC) curve analysis was applied ,it shows the diagnostic performance of serum MCP-1 in rheumatoid arthritis patients(active and inactive) versus the healthy control group, at a cut-off level of 52.5 ng/ml. Moreover the diagnostic sensitivity, specificity, negative predictive value and positive predictive value of 98%, 93%, 93% and 98% respectively .

ROC- curve analysis was applied to assess the diagnostic performance of serum MCP-1 in discriminating active rheumatoid arthritis patients from the inactive group, at a cut-off level of 61ng/ml. This had a diagnostic sensitivity, specificity, negative predictive value and positive predictive value of 98%, 93%, 93% and 98% respectively. These findings highlight the possible role of this marker (MCP-1) in discriminating active rheumatoid arthritis patients from the inactive disease.

RECOMMENDATIONS:

-The value of the marker (sMCP-1) in monitoring response to therapy is worth exploration.

-Further research studies are indicated to assess serum levels of the marker in a trial to evaluate their role in assessment of disease activity as compared to the estimated serum MCP-1 levels.

-Further studies should be done on large sample size to determine the predictive value of this biomarker for RA disease.

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