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Estimation of Autophage Function for Clearance of Hyperlipidemia with Concern of Anti-Rheumatoid Drugs

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ABSTRACT

Background: Dyslipidemia is the common complication in rheumatoid arthritis (RA) patient, and subsequently increased CVD risk. Dyslipidemia is the net result between positive intake of lipids and clearance by autophage.

Objective: The aim of this work is to evaluate the effect of autophages function as clearance of dyslipidemia in two different lines of active RA treatment. **Patients and methods:** This study was carried out at Agouza Police Hospital. It was analysis of data from one hundred and twenty individuals who were admitted to the hospital. Their age ranged from 40-65 years. They were 84 females (70%) and 36 males (30%) healthy and patient with rheumatoid arthritis attending the outpatient clinic and the inpatient of the Department of Rheumatology.

Results: There was significant increase of cholesterol in group 2, 3, and 4. There was significant increase of LDL in group 2 and 4 while it decreased in group 3 which showed significant increase in HDL. There was significant increase of disease activity (DAS-28) in group 2, 3, and 4. The autophage function showed significant increase in all groups and this could be secondary increase of phagocytosis through the action of cytokines and defect of digestion secondary to therapeutic drugs. **Conclusion:** Methotrexate is immunosuppressive drug could be the one of the famous immunosuppressive drugs in RA that is completely responsible for dyslipidemia.

Keywords: Autophage Function, Clearance Hyperlipidemia, Concern of Anti-Rheumatoid Drugs.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease with joint inflammation and autoantibody production as key elements of its pathogenesis. RA remains a heterogeneous syndrome in terms of clinical expression and long-term course, and different pathogenic pathways are likely to be differently activated in different patients or at least in different phases of the disease. In particular, the relative contribution of B lymphocytes appears greatly variable, as inferred at least by the existence of a seropositive and a zero negative subtype of RA [(1)]. The net effect on atherosclerosis of the drugs used to treat RA is unclear. Corticosteroids could increase the risk of atherosclerosis via deleterious effects on lipids, glucose metabolism, and blood pressure. However, corticosteroids could also decrease the risk of atherosclerosis by controlling inflammation. Equally, use of corticosteroids could just be an indicator of more severe RA [(2)].

In this context, novel biomarkers reflecting RA pathogenesis could be tested for improving the recognition and management of chronic arthritis since the very early phases of the disease, before the fulfillment of any established classification criterion[(3)].

RA is associated with an adverse lipid profile that is conventionally accepted as a risk factor for cardiovascular disease [(4)]. Lipid profile can be improved to an extent that is clinically meaningful by effectively treating RA without using a lipid lowering agent[(5)].

AIM OF THE WORK

The aim of this work is to evaluate the effect of autophages function as clearance of dyslipidemia in two different lines of active RA treatment.

SUBJECTS AND METHODS

This study was carried out at Agouza Police Hospital. It was analysis of data from one hundred and twenty individuals who were admitted to the hospital. Their age ranged from 40-65 years. They were 84 females (70%) and 36 males (30%) healthy and patient with rheumatoid arthritis attending the outpatient clinic and the inpatient of the Department of Rheumatology, Rehabilitation and Physical Medicine. They were divided into 4 groups:

Group 1: 30 healthy volunteers considered as negative control.

Group 2: 30 patient discovered early without medication had either moderate or high disease activity (DAS-28 >3.2) as a positive control.

Group 3: 30 patient taking combined therapy (immune suppressive + immunestimulant) and were going in remission or had low disease activity (DAS-28 <3.2).

Group 4: 30 patient taking immune suppressive medication (methotrexate) and were going in remission or had low disease activity (DAS-28 <3.2).

All patients included in the study met the American College of Rheumatology (ACR) revised criteria for classification of rheumatoid arthritis [(6)].

- ***Exclusion criteria:***

Patients and control were excluded from the study when they had:

- Tobacco smoking.
- Chronic diseases including (hypertension, diabetes or hypercholesterolemia).
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Ethical considerations

An informed consent was taken from patients included in the study and it was approved by Ethical

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Laboratory investigations:

1-Erythrocyte sedimentation rate.

2- C-reactive protein (hs-CRP) by auto analyzer, ARCHITECT ER-200.

3-Rheumatoid factor by auto analyzer, ARCHITECT ER-200.

4-Anticyclic citrullinated peptide (ACCP) antibodies by ELISA ®.

5-Lipid profile: including total serum cholesterol (SC), low density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C) and serum triglyceride level (TGs) were carried out on auto analyzer Beckman coulter AU 480.

6-Estimation of neutrophil lipid phagocytes by Sudan black stain: The percentage of active engulfing lipids in hundred cells was calculated.

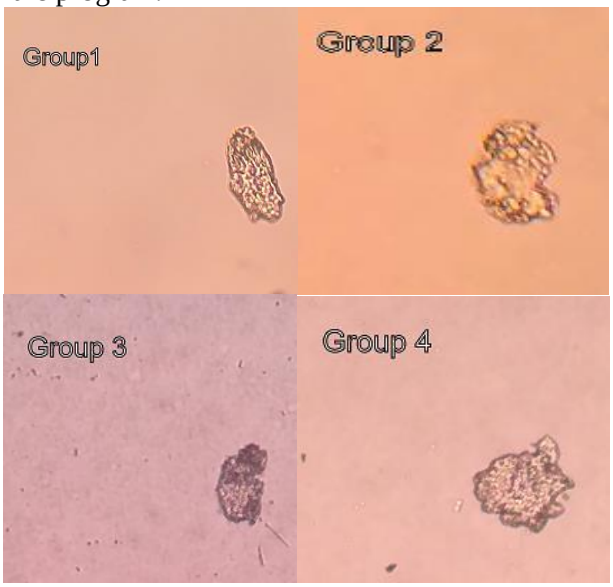
Method and principle of estimation

PRINCIPLE: Sudan black is slightly basic dye, which combines with acidic groups in compound lipids, thus staining phospholipids also [7].

Reagent: Sudan black solution (1 mg per 100 ml buffer phosphate saline) and Ca gluconate 1% to enhance the penetration of Sudan black into lipid containing cells.

Method:

- The plasma rich cells were used for staining and calculation
- The stain was warmed at 37°C before using it. Random hundred stained cells of each group were photographed and processing into image Photoshop program **(Image j free internet Photoshop program).**
- The amount of fat inside the selected cells was calculated by the program.



7- ASCVD (Atherosclerotic cardiovascular disease) 2013 risk calculator from AHA/ACC: determines 10 year risk of heart disease or stroke.

Statistical analysis

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

- Independent-samples t-test of significance was used when comparing between two means.
- Chi-square (x^2) test of significance was used in order to compare proportions between two qualitative parameters.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered significant as the following:

- P-value <0.05 was considered significant.
- P-value <0.001 was considered highly significant.
- P-value >0.05 was considered insignificant.

RESULTS

Table (1): statistical analysis for CRP between group 1 and the other three groups.

CRP	Mean	STD	T. Test	P. Value
Group 1	4.734	3.90062		
Group 2	30.59	27.30476	3.873	< 0.01
Group 3	8.402	10.23539	3.509	< 0.01
Group 4	5.57	3.5343	0.6756	>0.05

Table 1 showed significant increase in group 2 and 3 compared to group 1.

Table (2): statistical analysis for ESR between group 1 and the other three groups.

ESR	Mean	STD	T. Test	P. Value
Group 1	9.7	28.769		
Group 2	18.7	7.6891	3.873	< 0.01
Group 3	12.6	4.42719	3.509	< 0.01
Group 4	12.2	4.56557	3.509	< 0.01

Table 2 showed significant increase in group 2, 3 and 4 compared to group 1.

Table (3): statistical analysis for RF between group 1 and the other three groups.

RF	Mean	STD	T. Test	P. Value
Group 1	10.129	7.49566		
Group 2	80.77	99.10159	3.873	< 0.01
Group 3	20.392	17.94189	1.54898	>0.05
Group 4	31.718	22.83834	3.509	< 0.01

Table 3 showed significant increase in group 2 and 4 compared to group 1.

Table (4): statistical analysis for Anti-CCP between group 1 and the other three groups.

Anti- CCP	Mean	STD	T. Test	P. Value
Group 1	0.709	0.43093		
Group 2	68.801	79.48804	3.873	< 0.01
Group 3	29.3	28.09527	3.21466	< 0.01
Group 4	32.31	61.54356	3.509	< 0.01

Table 4 showed significant increase in group 2, 3, and 4 compared to group 1.

Table (5): statistical analysis for disease activity (DAS-28) between group 1 and other three groups.

DAS-28	Mean	STD	T. Test	P. Value
Group 1	0	0		
Group 2	3.74	0.22705	3.74	< 0.01
Group 3	2.15	0.65532	2.15	< 0.01
Group 4	2.34	0.57388	3.509	< 0.01

Table 5 showed significant increase of DAS-28 in group 2, 3, and 4 compared to group 1.

Table (6): statistical analysis for cholesterol between group 1 and the other three groups.

Cholesterol	Mean	STD	T. Test	P. Value
Group 1	181.7	31.62998		
Group 2	222.2	50.79764	3.509	< 0.01
Group 3	219.2	46.20197	2.4347	< 0.05
Group 4	242.5	74.10091	3.874	< 0.01

Table 6 showed highly significant increase in group 2, 3, and 4 compared to group 1.

Table (7): statistical analysis for LDL between group 1 and the other three groups.

LDL	Mean	STD	T. Test	P. Value
Group 1	96.06	37.33356		
Group 2	125.0	52.957	3.609	<0.01
Group 3	122.3	41.60141	0.1105	>0.05
Group 4	142.0399	63.49979	3.873	<0.01

Table 7 showed significant increase of LDL in serum in group 2 and 4 compared group 1.

Table (8): statistical analysis for HDL between group 1 and the other three groups.

HDL	Mean	STD	T. Test	P. Value
Group 1	48.9	14.40255		
Group 2	45.4	11.30585	3.5	>0.05
Group 3	60.0	16.88523	2.903	<0.05>
Group 4	41.6	7.29079	1.509	>0.05

Table 8 showed that HDL significantly increased in groups 3.

Table (9): statistical analysis for triglycerides between group 1 and the other three groups.

TGs	Mean	STD	T. Test	P. Value
Group 1	164.12	92.37		
Group 2	248.50	89.64	3.446	< 0.01
Group 3	207.20	52.75	3.509	< 0.01
Group 4	216.10	70.73	0.6756	>0.05

Table 9 showed triglycerides was significant increase in groups 2 and 3.

Table (10): statistical analysis for autophage index between group 1 and the other three groups.

Autophage index	Mean	STD	T. Test	P. Value
Group 1	154.9	28.769		
Group 2	154.7	34.7916	2.903	< 0.01
Group 3	189.0	28.06738	2.903	< 0.01
Group 4	225.5	15.11622	3.509	< 0.01

Table 10, showed significant increase in group 2, 3, 4 compared group 1.

Table (11): statistical analysis for risk of cardiovascular disease (CVD) between group 1 and the other three groups.

Risk of CVD	Mean	STD	T. Test	P. Value
Group 1	2.240	2.188		
Group 2	2.630	1.599	2.772	< 0.01
Group 3	6.180	3.473	3.509	0.01<
Group 4	10.250	8.500	3.873	< 0.01

Table 11, showed significant increase in group 4 compared to group 1.

DISCUSSION

The present study was done on 120 individuals; 90 of them had rheumatoid arthritis and 30 were apparently healthy control. The majority of the patients were females and this attributed to the disease prevalence.

In our study, patients with active RA showed dyslipidemia and had a more marked atherogenic lipid profile compared with the negative control group. This atherogenic lipid profile included significant increased total cholesterol and LDL except group 3.

The statistical results showed hypercholesterolemia in patient with active disease either with medication or not. Cholesterol was significant in all groups. This results agreed with **Johansen *et al.*** [8] **(**who found that patients with RA exhibited higher TC, LDL, HDL and TG compared to controls.

HDL was significantly increased in group 3 only while LDL was significantly decreased in group 3. This result can explain that the rise of cholesterol is due to HDL not LDL [5]**.** This could be the rule of hydroxychloroquine, which was useful for lowering total cholesterol and LDL but had no effect on HDL. This results agreed with **Desai *et al.*** [9]**.**

The level of TGs in group 1 and 2 was not affected this result give us the contention that RA activity has no rule on triglycerides. Subsequently, RA treatment has no effect on TGs. This result coincides with **Rodríguez-Carrio *et al.* [10].**

ESR, CRP and anti-CCP showed significant increase in group 2. This proves that the selected patients were in active state, while in groups 3 and 4 the difference was not significant. This was due to the action of therapeutic drugs.

In our study the action of RA disease is due to increased circulating immune-complex, complement and subsequence activation of immunological cells and release of proinflammatory and inflammatory cytokines.

Group 4 had normal ESR due to the action of methotrexate to stop the inflammatory cytokines. Proteins that affect the result of ESR are fibrinogen and beta 2 micro globulins. Methotrexate has a direct action to stop the release of proinflammatory cytokines (IL6 – IL1) **[11].**

DAS-28 score was significantly decreased in groups 3 and 4 compared to group 2. Methotrexate therapy with or without hydroxychloroquine had a significant decrease in DAS-28 score.

Autophage is a phagocytic cells supposed to be responsible for clearance of dyslipidemia. This action done by phagocytes precipitating lipids and digest the lipids inside phagosomes so as external factors regulate its phagocytic function. Inflammatory cytokines which are present in active stage in RA help for phagocytosis and digestion **[12].**

In our study on the other hand, immune suppressive drugs as methotrexate suppresses phagocytosis and digestion. The net result is that patient autophage would be facing balance between immunestimulant inflammatory mediators and immunosuppressive drugs (methotrexate).

From our results the lipid content in autophage was significantly increased in group 2, 3, and 4. The patient lipid inside the cells means active phagocytosis. But digestion could be completely lacked.

CONCLUSION

Methotrexate is immunosuppressive drug that could be the one, of the famous immunosuppressive drugs in RA, completely responsible for dyslipidemia.

RECOMMENDATION

We advise the RA patient to take immunostimulant in combination with methotrexate or using antilipidemic drugs in combination with methotrexate in RA patient especially in active state.

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