To study the patterns of lipoprotein lipase activities, concentrations and related metabolites in patients with Rheumatoid arthritis

Priya Saini[1*], Sujata Wangkheimayum[2], Varun Dhir[3]

[1]Department of Paramedical, SVFTM, Swami Vivekanand Institute of Engineering and Technology, Banur-140401, Disst – Patiala, Punjab
[2] Department of Biochemistry, PGIMER, Chandigarh,India, 160012
[3] Department of internal Medicines.PGIMER, Chandigarh,India, 160012

***Corresponding author**: - Priya Saini, priyabiotech222@gmail.com*

Abstract

Rheumatoid arthritis (RA) affects nearly 1% of our world population. The drug named Methotrexate (Mtx), acts as a folate antagonist and is commonly used to treat rheumatoid arthritis. Lipoprotein lipase (LPL), an enzyme, which is present in endothelial lining is involved in the hydrolysis of triglycerides rich lipoproteins and producing free fatty acid (FFA) with glycerol. Our primary focus was to study the role of Lipoprotein Lipase (LPL) in Rheumatoid arthritis.

Materials and Methods

A total of 40 healthy control, 20 naive RA patients and 20 methotrexate treated RA patients were taken for the study. LPL activity and its concentration were checked by manual, chemical methods and ELISA. Lipid profile and other biochemical parameters were performed in auto-analyzer.

Conclusion

We found LPL concentration and its activity were lower in RA patients as compared to healthy controls ($p<0.05$) But no significant difference were seen in between naive and methotrexate treated RA patients. There were increased levels of LDL, TG and cholesterol in RA patients and showed no difference in HDL. An unfavorable lipid profile could be due to lower LPL enzyme activity in RA patients.

Key words

Rheumatoid Arthritis (RA), Lipoprotein Lipase (LPL), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Triglycerides (TG).

Introduction

Rheumatoid arthritis (RA) is an autoimmune disorder, characterized by chronic inflammation affecting joints. It results warm, swollen, and painful joints. Most commonly, the wrist and hands are involved, with the same joints typically involved on both sides of the body. The disease may also affect other parts of the body. It is prevalent in all corners of the globe with higher incidence in females. Genetic as well as environmental factors are all implicated in the disease. If not treated, chronic inflammation leads to cardiovascular disease and is reported to be the main cause of death in RA [1]. Modifications in structural and functional properties of lipoproteins producing atherogenic lipoproteins have been observed by some researchers [2]. Others have reported changes in lipid profile and acute phase reactants promoting atherosclerosis at an early stage [3]. Lipoprotein lipase (LPL) catalyses the hydrolysis of the triacylglycerol component of circulating chylomicrons and very low density lipoproteins, thereby providing non-esterified fatty acids and 2-monoacylglycerol for tissue utilization. LPL function have been found to be associated with a number of pathophysiological conditions, including atherosclerosis, chylomicrons, obesity, Alzheimer's disease, and dyslipidaemia associated with diabetes, insulin resistance, and infection [4]. The LPL gene is located on chromosome 8p22, spans ~30 kb and is divided into 10 exons, and has substantial sequence homology among most of the species that have been examined [5]. Almost 100 naturally occurring mutations in the LPL gene have been described in humans. There are 61 missense mutations, most of which are located on exons 5 and 6; 12 nonsense mutations, 10 frameshift mutations or small insertions/deletions, 3 gross mutations, 8 splicing mutations, and 4 promotor variants

Lipoprotein lipase (LPL) is present in the endothelial lining of the adipose tissue, heart, lungs and many tissue. In adult liver, it is not active [6]. It is a rate limiting enzyme and hydrolyses the portion of triglycerides present in chylomicrons and VLDL to liberate free fatty acids (FFA) and glycerol. Deficiency of it leads to impaired lipid profile with risks of CVD. Not much data are available for LPL RA patients. Therefore, the present study aims to investigate changes, if any, of these enzymes in patients with Rheumatoid arthritis. Correlation studies with related metabolites will also be carried out.

Materials and Methods:

Study Population: A total of 40 control subjects and 40 patients with RA [20 naive and 20 on treatment of at least for 4- 6 months mainly methotrexate] of both genders were taken for the study after obtaining informed consent. About 4 to 5 mL of blood were collected in the morning after an overnight fast.

We matched 1 healthy control with 1 RA with age difference of ±3 years within same gender. A detailed history of patients with RA including duration of disease, number of joints involved, medication, family history and other relevant information were obtained. Their height, weight, waist, hip and mid arm circumference was measured to the nearest cm or kg.

The waist circumference was measured at the midpoint between the lower border of the ribcage and iliac crest, whereas the hip circumference was obtained at the widest point between hip and buttock. Accordingly, their BMI(body mass index) was calculated from their height and weight measurements. The non-obese subjects were classified into the normal weight (BMI≥18.5 and <25) and overweight (BMI≥25 and <30) groups and control subjects and patients of two different age groups were classified within each BMI category [85].

LPL activity, glycerol, were performed manually by chemical methods. In this study we investigated the patterns of lipoprotein lipase activities, concentrations and related metabolites in three different groups of healthy subject, RA patients, before and after treatment. LPL mass was carried out by gel electrophoresis and quantitated. Concentration was estimated by a commercial ELISA kit. All other parameters were assayed in autoanalyzers.

Data were analyzed by SPSS for Windows. Student's t test,Mann Whitney test, one way ANOVA, KruskalWallis test, post hoct test, correlation and regression studies, etc., were also carried out.

BIOCHEMICAL ANALYSES: LPL Activity is estimated based on the determination of glycerol, the product of LPL hydrolysis. The rate of hydrolysis of glycerol was estimated and enzyme activity was calculated. Futher, we had checked LPL concentration through Human Lipoprotein Lipase Elisa Kit-This test was done using ELISA kit (human lipoprotein lipase ELISA kit) from Bioassay Technology Laboratory and SDS PAGE.

Results

Glycerol concentration

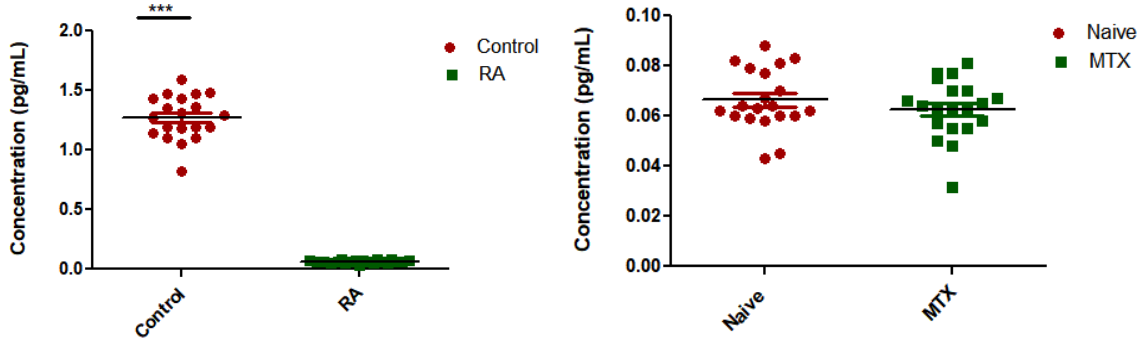

Figure 1: Lower glycerol concentration has shown in RA patients as compared to healthy control

LPL Activity

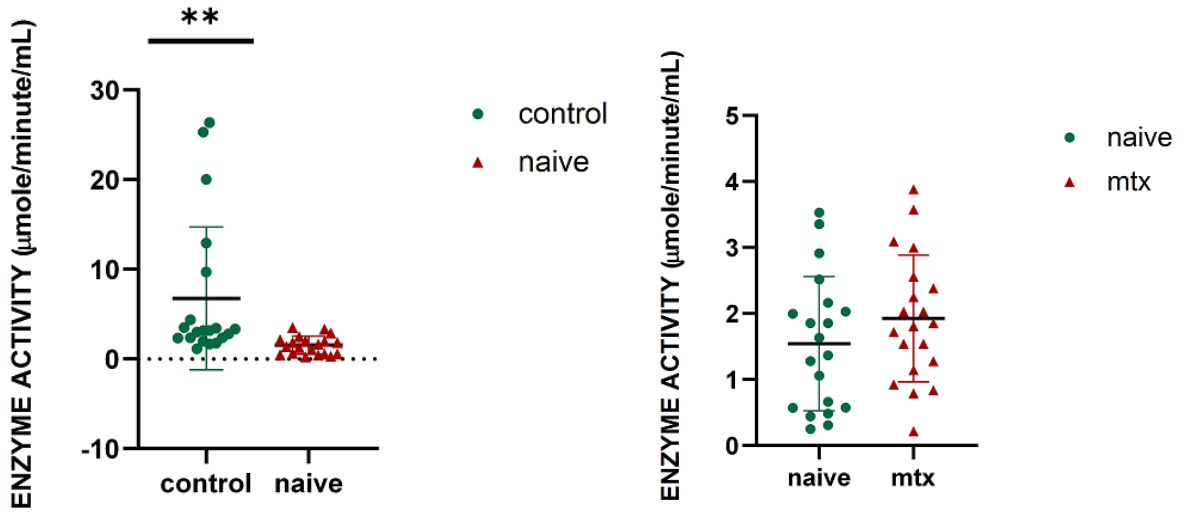

Figure 2: Lower LPL activity has shown in diseased condition

LPL Concentration

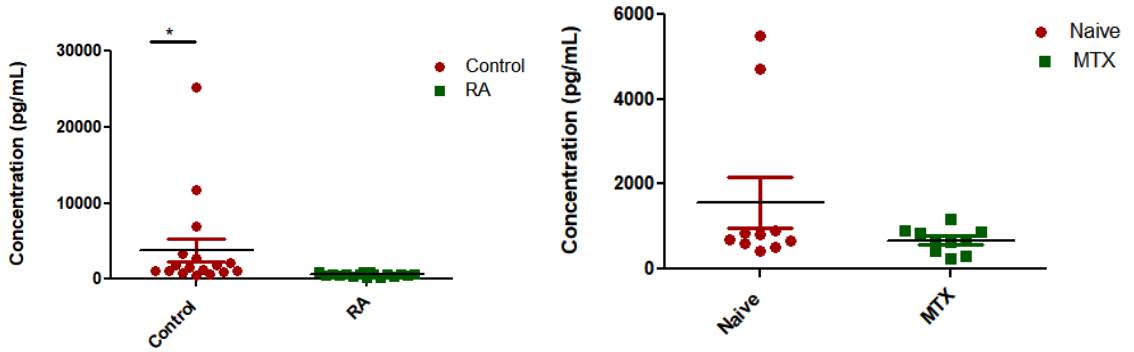

Figure 3: Slightly lower LPL concentration was found in RA patients as compared to healthy control

Low density lipoprotein (LDL)

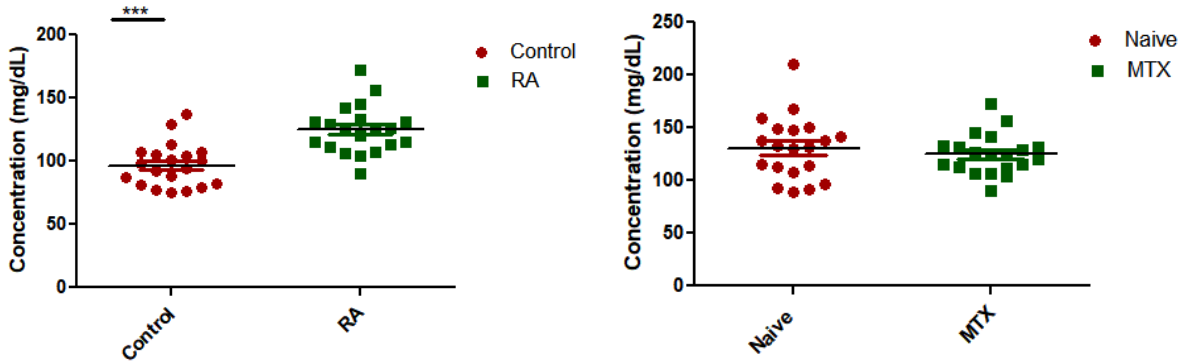

Figure 4: LDL level found to be elevated in diseased condition as compared to healthy control

High density lipoprotein (HDL)

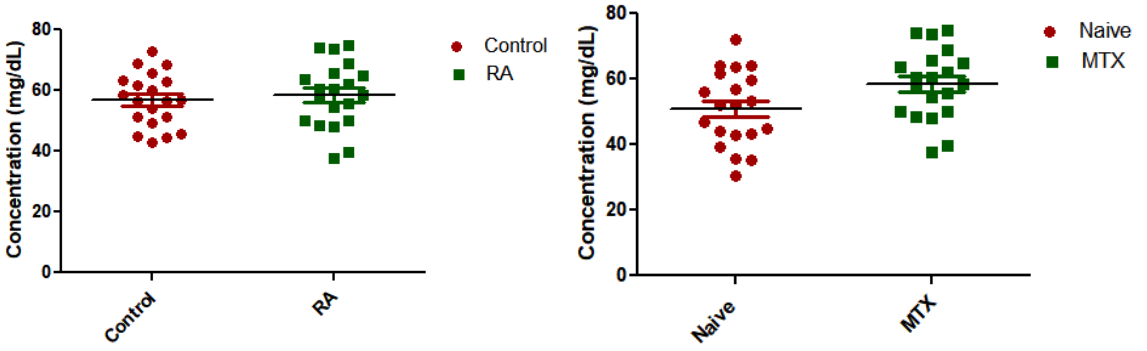

Figure 5: There is no significant difference in level of HDL

Triglyceride (TG)

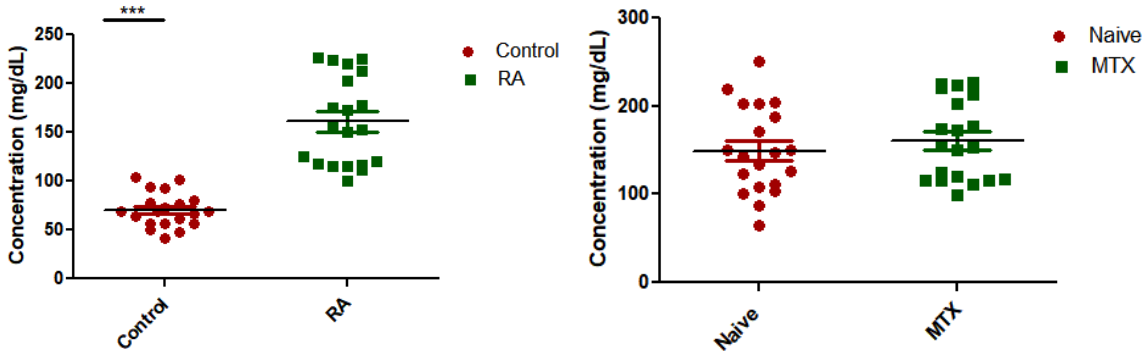

Figure 6: Triglycerides was found to be more in RA patients as compared to control

Cholesterol

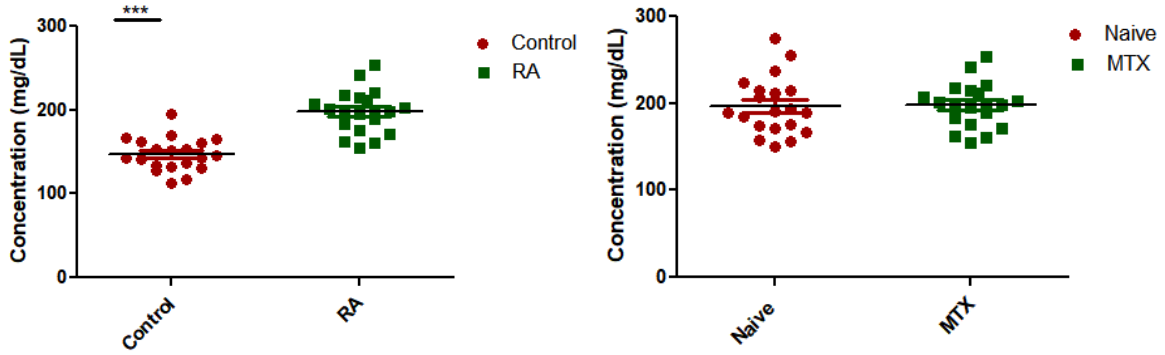

Figure 7: Cholesterol are also seem to be elevated in RA patients as compared to control

Table 1. Demographic Profile of Study Group

Parameters	Control (20)	Naïve (20)	Mtx (20)	Significance, P value
Age	23.30 ± 2.02	42.75 ± 10.40	46.55 ± 10.92	<.OO1***
BMI (kg/m^2)	22.47 ± 3.43	23.90 ± 3.76	25.53 ± 4.29	<.05*

Values are expressed as $mean \pm SD$

Figures in parentheses indicate n, number

P<0.05* is considered as statistically significant and p<0.001*** is considered as highly significant

ns= not significant
n= no. of subjects

Table 2. Comparison between control and diseased
Table 2.1. Non parametric data: Mann-Whitney Test

Parameters	Control (n=20)	RA Patients (n=20)	Significance, P value
Glycerol conc (µg/dL).	$1.27 \pm .18$	$.11 \pm .31$	<.001***
LPL Activity (µmoles/minute/mL)	6.7 ± 7.97	$1.7346 \pm .99540$	<.001***
LPL conc. (pg/mL)	3286.54 ± 5829.052	1170.94 ± 238.53	<.05*
AST (U/L)	16.42 ± 3.56	26.70 ± 9.45	<.001***
Conjugated bilirubin (mg/dL)	$.16 \pm .13$	$.15 \pm .07$	<.05*
Total protein (g/dL)	$7.50 \pm .25$	$8.45 \pm .87$	<.001***
Albumin (g/dL)	$4.59 \pm .19$	4.78±0.39	.577
Lipase (U/L)	36.05 ± 9.85	34.27 ± 16.00	.410

Values are expressed as $mean \pm SD$

Figures in parentheses indicate n, number
P<0.05* is considered as statistically significant and p<0.001*** is considered as highly significant

ns= not significant
n= no. of subjects

Table 2.2. Parametric data: T test

Parameters	Control (20)	RA Patients (20)	Significance,P value
Creatinine (mg/dL)	.58 ±.064	.79 ±1.42	<.001***
Uric acid (mg/dL)	3.40 ± .147	4.97 ± 1.13	<.001***
ALP (U/L)	68.80 ±17.46	96.40 ± 25.33	<.001***
ALT (U/L)	15.44 ±8.25	24.99 ± 11.99	<.001***
Total bilirubin (mg/dL)	.47 ±.17	.43 ±.20	.563
Cholesterol (mg/dL)	146.88 ±19.85	197.33±29.50	<.001***
LDL (mg/dL)	96.64 ±17.11	127.90 ±25.00	<.001**
HDL (mg/dL)	56.82 ±8.80	50.88 ± 11.39	.463
TG (mg/dL)	70.37 ±17.42	155.01 ±47.02	<.001***

Values are expressed as $mean \pm SD$

Figures in parentheses indicate n, number
P<0.05* is considered as statistically significant and p<0.001*** is considered as highly significant

ns= not significant
n= no. of subjects

Table 3. Comparison between control-naïve-Mtx
Table 3.1. Non parametric data: Kruskal-Wallis Test

Comparison of the biochemistry parameters between the 3 study groups

Parameters	Control(20)	Naïve(20)	Mtx (20)	Significance, P value
Glycerol conc (µg/dL).	1.27 ± .18	.06 ± .01	.16 ± .44	<.001***
LPL Activity (µmoles/minute/mL)	6.74 ± 7.97	1.54 ± 1.01	1.92 ± .96	<.001***
LPL conc. (pg/mL)	3286.54 ± 5829.05	1567.48 ± 1877.08	799.58 ± 465.67	<.05*
Urea (mg/dL)	16.93 ± 3.93	28.47 ± 6.28	25.08 ± 6.52	<.001***
AST (U/L)	16.42 ± 3.56	23.47 ± 9.69	29.93 ± 8.21	<.001***
Conjugated bilirubin (mg/dL)	.16 ± .13	.12 ± .06	.18 ± .07	.089
Total protein (g/dL)	7.50 ± .25	8.20 ± .73	8.70 ± .94	<.001***
Albumin (g/dL)	4.59 ± .19	4.53 ± 0.40	5.04 ± .39	<.001***

Lipase (U/L)	36.05 ± 9.85	37.35 ± 19.52	31.34 ± 11.51	.542

Values are expressed as $mean \pm SD$

Figures in parentheses indicate n, number

P<0.05* is considered as statistically significant and p<0.001*** is considered as highly significant

ns= not significant,n= no. of subjects

Table 3.2. Parametric data: One way Anova

Comparison of the biochemistry parameters between the 3 study groups

Parameters	**Control (20)**	**Naïve (20)**	**Mtx (20)**	**Significance, P value**
Creatinine (mg/dL)	.5835 ± .06418	.7511 ± .10614	.8365 ± .16152	<.001***
Uric acid (mg/dL)	3.40 ± .66	4.69 ± 1.04	5.26 ± 1.17	<.001***
ALP (U/L)	68.80 ± 17.46	88.75 ±26.46	104.05 ± 22.22	<.001***
ALT (U/L)	15.44 ± 8.25	21.1450 ± 12.13	28.84 ± 10.82	<.001***
Total bilirubin (mg/dL)	.47 ± .17	.37 ± .13	.50 ± .25	.078
Cholesterol (mg/dL)	146.88 ± 19.85	196.97 ± 33.20	197.74 ± 25.88	<.001***
LDL (mg/dL)	96.64 ± 17.11	130.77 ± 29.98	125.04 ± 19.13	<.001***
HDL (mg/dL)	56.82 ± 8.80	50.88 ± 11.39	58.42 ± 10.63	.060
TG (mg/dL)	70.37 ± 17.42	149.17 ± 49.40	160.86 ± 45	<.001***

Values are expressed as $mean \pm SD$

Figures in parentheses indicate n, number

P<0.05* is considered as statistically significant and p<0.001*** is considered as highly significant

ns= not significant
n= no. of subjects

Table 4. Comparison between control-naïve, control-mtx, naive-mtx
Table 4.1.1. Non-Parametric data: Mann-Whitney Test

Comparison of biochemistry parameters between control and naïve RA patients

Parameters	**Control (20)**	**Naïve (20)**	**Significance, P value**
Glycerol conc (µg/dL).	$1.27 \pm .18$	$.06 \pm .01$	<.001***
LPL Activity (µmoles/minute/mL)	6.74 ± 7.97	1.54 ± 1.01	<.001***
LPL conc. (pg/mL)	3286.54 ± 5829.05	1567.48 ± 1877.08	<.05*
Urea (mg/dL)	16.93 ± 3.93	28.47 ± 6.28	<.001***
AST (U/L)	16.42 ± 3.56	23.47 ± 9.69	<.05*
Conjugated bilirubin (mg/dL)	$.16 \pm .13$	$.12 \pm .06$	.506
Total protein (g/dL)	$7.50 \pm .25$	$8.20 \pm .73$	<.001***
Albumin (g/dL)	$4.59 \pm .19$	4.53 ± 0.40	.989
Lipase (U/L)	36.05 ± 9.85	37.35 ± 19.52	.899

Values are expressed as $mean \pm SD$

Figures in parentheses indicate n, number

P<0.05* is considered as statistically significant and p<0.001*** is considered as highly significant

ns= not significant

Table 4.1.2. Non-Parametric data: Mann-Whitney Test

Comparison of the biochemistry parameters between control and treated RA patients

Parameters	**Control (20)**	**Mtx (20)**	**Significance, P value**
Glycerol conc (µg/dL).	$1.27 \pm .18$	$.16 \pm .44$	<.001***
LPL Activity (µmoles/minute/mL)	6.74 ± 7.97	$1.92 \pm .96$	<.001***
LPL conc. (pg/mL)	3286.54 ± 5829.05	799.58 ± 465.67	<.05*
Urea (mg/dL)	16.93 ± 3.93	25.08 ± 6.52	<.001***
AST (U/L)	16.42 ± 3.56	29.93 ± 8.21	<.001***

Conjugated bilirubin (mg/dL)	$.16 \pm .13$	$.18 \pm .07$	.103
Total protein (g/dL)	$7.50 \pm .25$	$8.70 \pm .94$	<.001***
Albumin (g/dL)	$4.59 \pm .19$	$5.04 \pm .39$	<.001***
Lipase (U/L)	36.05 ± 9.85	31.34 ± 11.51	.204

Values are expressed as $mean \pm SD$

Figures in parentheses indicate n, number

P<0.05* is considered as statistically significant and p<0.001*** is considered as highly significant

ns= not significant
n= no. of subjects

Table 4.1.3. Non-Parametric data: Mann-Whitney Test
Comparison of the biochemistry parameters between naïve and treated RA patients

Parameters	**Naïve (20)**	**Mtx (20)**	**Significance, P value**
Glycerol conc (µg/dL).	$.06 \pm .01$	$0.16 \pm .44$	.828
LPL Activity (µmoles/minute/mL)	1.54 ± 1.01	$1.92 \pm .96$	.223
LPL conc. (pg/mL)	1567.48 ± 1877.08	799.58 ± 465.67	.792
Urea (mg/dL)	28.47 ± 6.28	25.08 ± 6.52	<.05*
AST (U/L)	23.47 ± 9.69	29.93 ± 8.21	<.05*
Conjugated bilirubin (mg/dL)	$0.12 \pm .06$	$.18 \pm .07$	<.05*
Total protein (g/dL)	$8.20 \pm .73$	$8.70 \pm .94$	.130
Albumin (g/dL)	4.53 ± 0.40	$5.04 \pm .39$	<.05*
Lipase (U/L)	37.35 ± 19.52	31.34 ± 11.51	.593

Values are expressed as $mean \pm SD$

Figures in parentheses indicate n, number
P<0.05* is considered as statistically significant and p<0.001*** is considered as highly significant

ns= not significant
n= no. of subjects

Table 4.2.1. Parametric data: Post Hoc Tests

Comparison of the biochemistry parameters between control and naïve RA patients

Parameters	**Control (20)**	**Naïve (20)**	**Significance, P value**
Creatinine (mg/dL)	$.5835 \pm .06418$	$.7511 \pm .10614$	<.001***
Uric acid (mg/dL)	$3.40 \pm .66$	4.69 ± 1.04	<.001***
ALP (U/L)	68.80 ± 17.46	88.75 ± 26.46	<.05*
ALT (U/L)	15.44 ± 8.25	21.1450 ± 12.13	.276
Total bilirubin (mg/dL)	$.47 \pm .17$	$.37 \pm .13$	.322
Cholesterol (mg/dL)	146.88 ± 19.85	196.97 ± 33.20	<.001***
LDL (mg/dL)	96.64 ± 17.11	130.77 ± 29.98	<.001***
HDL (mg/dL)	56.82 ± 8.80	50.88 ± 11.39	.224
TG (mg/dL)	70.37 ± 17.42	149.17 ± 49.40	<.001***

Values are expressed as $mean \pm SD$

Figures in parentheses indicate n, number
P<0.05* is considered as statistically significant and p<0.001*** is considered as highly significant ns= not significant

n= no. of subjects

Table 4.2.2. Parametric data: Post Hoc Tests

Comparison of the biochemistry parameters between control and Mtx treated RA patients

Parameters	**Control (20)**	**Mtx (20)**	**Significance, P value**
Creatinine (mg/dL)	$.5835 \pm .06418$	$.8365 \pm .16152$	<.001***
Uric acid (mg/dL)	$3.40 \pm .66$	5.26 ± 1.17	<.001***
ALP (U/L)	68.80 ± 17.46	104.05 ± 22.22	<.001***
ALT (U/L)	15.44 ± 8.25	28.84 ± 10.82	<.001***
Total bilirubin (mg/dL)	$.47 \pm .17$	$.50 \pm .25$	1
Cholesterol (mg/dL)	146.88 ± 19.85	197.74 ± 25.88	<.001***
LDL (mg/dL)	96.64 ± 17.11	125.04 ± 19.13	<.001***
HDL (mg/dL)	56.82 ± 8.80	58.42 ± 10.63	1
TG (mg/dL)	70.37 ± 17.42	160.86 ± 45	<.001***

Values are expressed as $mean \pm SD$

Figures in parentheses indicate n, number

P<0.05* is considered as statistically significant and p<0.001*** is considered as highly significant

ns= not significant
n= no. of subjects

Table 4.2.3. Parametric data: Post Hoc Tests (Comparison of the biochemistry parameters between RA patients (naïve vs treated)

Parameters	**Naïve (20)**	**Mtx (20)**	**Significance, P value**
Creatinine (mg/dL)	$.7511 \pm .10614$	$.8365 \pm .16152$	.082
Uric acid (mg/dL)	4.69 ± 1.04	5.26 ± 1.17	.216
ALP (U/L)	88.75 ± 26.46	104.05 ± 22.22	.104
ALT (U/L)	21.1450 ± 12.13	28.84 ± 10.82	.073
Total bilirubin (mg/dL)	$.37 \pm .13$	$.50 \pm .25$	.089
Cholesterol (mg/dL)	196.97 ± 33.20	197.74 ± 25.88	1
LDL (mg/dL)	130.77 ± 29.98	125.04 ± 19.13	1
HDL (mg/dL)	50.88 ± 11.39	58.42 ± 10.63	.074
TG (mg/dL)	149.17 ± 49.40	160.86 ± 45	1

Values are expressed as $mean \pm SD$

Figures in parentheses indicate n, number

P<0.05* is considered as statistically significant and p<0.001*** is considered as highly significant

ns= not significant
n= no. of subjects

Table 5. Correlation between LPL and the related biochemical parameters of control (n=20)

Parameters	**Correlation Coefficient, r**	**Significance, P value**
LPL Activity and glycerol	.034	.886
LPL Activity and cholesterol	-.143	.548
LPL Activity and LDL	-.033	.890
LPL Activity and HDL	-.029	.905
LPL Activity and TG	-.160	.500

Correlation is significant at the 0.01 level

Correlation is significant at the 0.05 level

Table 6. Correlation between LPL and related biochemical parameters of diseased

Parameters	**Correlation Coefficient, r**	**Significance, P value**
LPL Activity and glycerol	.467	<.001***
LPL Activity and cholesterol	-.005	.976
LPL Activity and LDL	-.062	.704
LPL Activity and HDL	.042	.799
LPL Activity and TG	.012	.941

Correlation is significant at the 0.01 level
Correlation is significant at the 0.05 level

Table 7. Correlation between BMI and related biochemical parameters of control

Parameters	**Correlation Coefficient, r**	**Significance, P value**
BMI and glycerol conc.	-.122	.610
BMI and LPL Activity	-.148	.534
BMI and LPL Conc.	.405	.077

Correlation is significant at the 0.01 level
Correlation is significant at the 0.05 level

Table 8. Correlation between BMI and related biochemical parameters of Diseased

Parameters	**Correlation Coefficient, r**	**Significance, P value**
BMI and glycerol conc.	-.038	.817
BMI and LPL Activity	-.104	.524
BMI and LPL Conc.	-.144	.446

Correlation is significant at the 0.01 level
Correlation is significant at the 0.05 level

Table 9. Reference range of biochemical parameters of healthy control

Parameters	**5th percentile**	**95th percentile**
Age	21.05	28.00
BMI (kg/m2)	16.820	29.630

Glycerol conc (µg/dl)	.835	1.582
LPL Activity (µmoles/min/ml)	1.175	26.315
LPL conc. (pg/mL)	133.626	24519.694
Creatinine (mg/dL)	.425	.690
Urea (mg/dL)	10.735	23.850
Uric acid (mg/dL)	2.400	4.770
ALP (U/L)	44.050	97.950
AST (U/L)	8.815	24.020
ALT (U/L)	.995	32.330
Tot. Bill (mg/dL)	.173	.785
Conj Bill (mg/dL)	.013	.680
Tot. protein (g/dL)	6.989	7.945
Albumin (g/dL)	4.341	4.920
Lipase (U/L)	22.315	56.980
Cholesterol (mg/dL)	112.525	194.485
LDL (mg/dL)	75.520	136.440
HDL (mg/dL)	42.885	72.785
TG (mg/dL)	42.120	103.805
Amylase (U/L)	35.500	73.950
Glucose (mg/dL)	65.640	110.945

Table 10. Reference range of biochemical parameters of naive group

Parameters	**5th percentile**	**95th percentile**
Age	25.10	58.95
BMI (kg/m2)	16.875	29.170
Glycerol conc (µg/dl)	.043	.088
LPL Activity (µmoles/min/ml)	.255	3.524
Urea (mg/dL)	18.715	39.275
Uric acid (mg/dL)	3.105	6.485
ALP (U/L)	16.600	151.200
AST (U/L)	9.765	51.740
ALT (U/L)	7.425	49.140
Tot. Bill (mg/dL)	.173	.649
Conj Bill (mg/dL)	.001	.220
Tot. protein (g/dL)	7.090	9.776
Albumin (g/dL)	3.922	3849.664
Cholesterol (mg/dL)	150.020	274.170
LDL (mg/dL)	89.030	208.615
HDL (mg/dL)	30.740	71.800
TG (mg/dL)	65.155	249.135

Glucose (mg/dL)	9.660	279.050

Table 11. Reference range of biochemical parameters of MTX group

Parameters	**5th percentile**	**95th percentile**
Age	32.00	71.80
BMI (kg/m2)	15.740	32.245
Glycerol conc (µg/dl)	.048	1.934
LPL Activity (µmoles/min/ml)	.250	3.871
LPL conc. (pg/mL)	238.941	2173.022
Creatinine (mg/dL)	.660	1.277
Urea (mg/dL)	19.500	43.115
Uric acid (mg/dL)	3.145	8.320
ALP (U/L)	53.250	139.900
AST (U/L)	18.910	50.175
ALT (U/L)	18.610	65.180
Tot. Bill (mg/dL)	.223	1.196
Conj Bill (mg/dL)	.101	.387
Tot. protein (g/dL)	7.702	10.469
Albumin (g/dL)	4.448	6.068
Lipase (U/L)	4.520	53.140
Cholesterol (mg/dL)	154.815	253.590
LDL (mg/dL)	91.285	171.795
HDL (mg/dL)	37.900	74.850
TG (mg/dL)	100.395	226.640
Amylase (U/L)	40.550	183.800
Glucose (mg/dL)	77.885	143.530

DENSITOMETRIC ANALYSIS OF SDS PAGE

SDS PAGE was run for serum samples of two groups and LPL enzyme band which is 52KDa enzyme was first isolated from SDS PAGE and then quantified using GEL-DOC. Different dilutions of samples were compared and 1:10 dilution of serum was used.

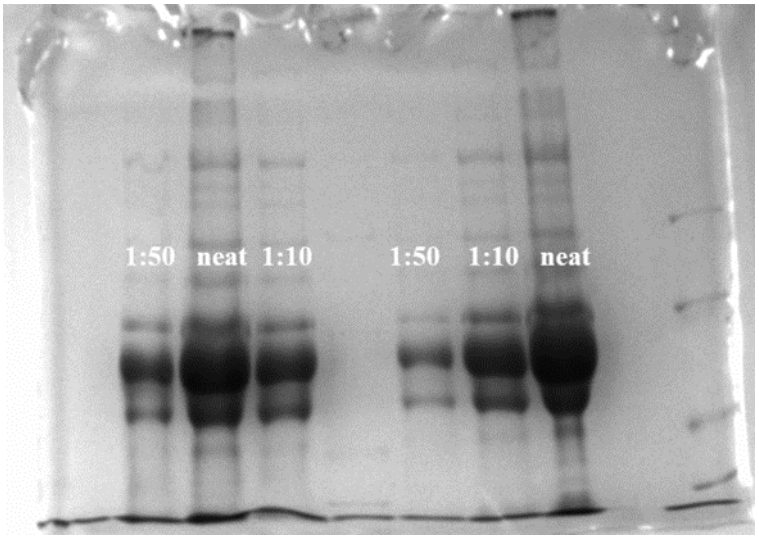

Figure 8: Different dilutions of serum done 1:10 dilution, no dilution , 1:50 dilution

Discussion

The demographic characteristics of the study groups are presented in table 1, as can be seen, there are significant difference in age and BMI among the three groups as assessed by one way ANOVA. As far as age is concerned, the RA patients (both naïve and treated) were the range of 40-50 years. The control subjects had a mean age of slightly less than 25 years.

Comparison between the biochemistry parameters of control subjects and RA patients (naïve and treated) were performed by Mann Whitney test for non-parametric data and student's T-test for parametric data, as provided in table.2.1. There are significant decrease in the glycerol concentration, LPL activity, LPL concentration, Direct bilirubin in the RA patients compared to the control subjects.

From table.2.1 and 2.2, we can see higher levels of urea, creatinine, uric acid, AST, ALT, ALP, cholesterol, TG, LDL in RA patients compared to control subjects. RA patients in our study are older and most are under some medication. Hence, the change in LFT (liver function test) and RFT (renal function test) are not surprising. The lower level of LPL could account for the increased level of cholesterol, TG, and LDL. It has been reported in literature, that LPL is lower in RA patients.

From table.3.1, we see that except for direct bilirubin and lipase, there are significant difference of the biochemical parameters between the 3 groups (control subjects, naïve RA patients, treated RA patients). Again except for total bilirubin and HDL, there were significant difference in the parameters between the 3 groups (table.3.2)

Table.4.1.1. shows the comparison of the parameters between control and drug-naïve RA patients. There is a significant decrease in glycerol concentration, LPL concentration, LPL activity in RA patients. The decrease in glycerol could be attributed to the decrease in LPL enzyme. The finding of a decreased LPL protein and enzyme activity is important and further

studies are necessary to find out the reason behind the change. Similar finding are observed between control subjects and methotrexate treated RA patients (table.4.1.2) From table 4.1.3, we see that AST, Direct bilirubin are significantly higher whereas, urea is lower in methotrexate treated RA patients as compared to naïve RA patients.

Compared to control, naïve RA patients had higher creatinine, uric acid, ALP, TG, LDL, and cholesterol (table.4.2.1). similar finding as above, and increased ALT was observed in mtx treated RA patients as compared to control subjects (table.4.2.2). There are significant decrees between naïve RA and MTX treated RA patents. As far as the routine biochemistry parameters are concerned (table 4.2.3).

As provided in table 5 there was no correlation between LPL activity and glycerol, as well as between LPL activity and lipid profile. In RA patients we noticed a significant correlation LPL and glycerol (table 6). Correlations between BMI and the parameters, glycerol, LPL activity, LPL concentration, did not yield any significant values in both the control and RA patients (table 7 and 8 respectively).

Table 9, 10, 11 are the reference ranges of biochemistry parameters of control, naïve RA patients, and MTX treated RA patients respectively. The values are presented as 5^{th} and 95^{th} percentiles as the data are non-parametric. Though this may be useful for future studies, the number of study subjects are few.

Graphic picture of glycerol, LPL activity, LPL concentration, LDL, HDL, TG and cholesterol are presented in figure 1, 2, 3, 4, 5, 6 and 7.

Figure 8 showing the appropriate bands could be seen at 1:10 dilution of sera, so further gel was run using 1:10 dilutions of the sera.

It is found that LPL concentration is lower in diseased condition as compared to healthy control.

From the above findings, we can conclude that in our study group (as in earlier studies) lipid profile shows an unfavourable trend with a higher cholesterol, TG, and LDL and lower HDL levels. As documented in literature, moderate increase of TG levels contribute to increased in risk of CVD (46).

Between the naïve RA patients and mtx RA patients, there is as significant increase in HO-1 conc as well as direct bilirubin conc is the latter. This could be a protected/ compensatory response. AST was increased and urea decreased in the treated group. However, these we still within the reference ranges of health. ALP was significantly increased in naïve RA patients compared to control and mtx RA patients had still higher levels of ALP than naïve RA patients.

That LFT and RFT parameters are increased (though within reference ranges) assumes clinical significance as the diseased process ad medication (5) could be responsible for the changes.

The reference values of non routine biochemistry parameters, not only of healthy controls, but of RA patients, separately, for naïve and drug- treated groups provided in this work would be very useful for future reference.

Conclusion

Rheumatoid arthritis (RA) is an autoimmune disorder, characterized by chronic inflammation affecting joints. It is prevalent in all corners of the globe with higher incidence in females. Genetic as well as environmental factors are all implicated in the disease. If not treated, chronic inflammation leads to cardiovascular disease and is reported to be the main cause of death in RA (1).

Lipid abnormalities have been shown in patients with rheumatoid arthritis, it has been reported that active and untreated RA showed a proatherogenic lipid profile, with a decrease in high-density lipoprotein cholesterol (HDL-C) being a more convincing finding [21].]. In RA, higher levels of LDL particles and lower levels of HDL particles compared with controls have been reported [24].

LPL are important enzymes involved in the metabolism of and lipids.

In the present study the following observation are noted :-

Glycerol concentration are significantly lower in RA patients as compared to healthy control. LPL activity and LPL concentration levels are also lower in patients having rheumatoid arthritis as compared to control

Urea, uric acid, creatinine are shown higher in diseased condition. .Though the values are still within the reference ranges of healthy subjects, they are signifant and contribution by the disease process, medications and other factors need to be worked out.

Liver function enzymes ALP, ALT, AST are also elevated in RA patients as compared to control. Again, the effects of the disease and medications need to be taken into consideration. Deranged lipid profile are obtained with significantly increased level of LDL, TG, cholesterol and decreased level of HDL in RA patients as compared to healthy control. These are in line with the decreased levels of LPL. The changes may contribute to an increased risk of CVD. Reference values of some non-routine parameters of healthy controls, drug-naïve RA patients and methotrexate treated RA patients would still be useful for future reference. However, more number need to be studied for well validated values.

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