Original Article

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A Study on Interleukin —1β and Lipid Profile as Markers of Cardiovascular Risk in Rheumatoid Arthritis

Biochemistry Section

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ABSTRACT

Introduction: The dyslipidaemia in Rheumatoid Arthritis (RA) is associated with accelerated atherosclerosis. A prospective clinical evaluation study was undertaken to find out the proportion of the rheumatoid arthritis patients who were suffering from dyslipidaemia, the change in the lipid levels and the disease activity after an intervention with antirheumatic therapy.

Aims and Objectives: To study the disease activity in Rheumatoid arthritis patients by measuring the serum levels of interleukin-1β (IL–1β), to find out the proportion of rheumatoid arthritis patients who were suffering from dyslipidaemia, to correlate the disease activity with the lipid profile and to look for the change in the lipid levels and the disease activity after an intervention with antirheumatic therapy.

Material and Methods: This study was done on 30 RA patients (fulfilling the American College of Rheumatology criteria). The lipid profile estimation was done by an enzymatic, colourimetric method and IL–1β was estimated by a chemiluminescence method. Dyslipidaemia was defined by taking the cut–off values of the NCEP–ATPIII guidelines. The patients with other comorbid illnesses and those who were on statins were excluded. The patients were followed up after 12 weeks of starting with the anti–rheumatic therapy.

Results: 36.7% of the patients had high total cholesterols, 53.3% of the patients had high triacylglycerol levels, 73.3% of the patients had decreased HDL–cholesterol and 33.3% of the patients had high LDL–cholesterols. 86.7% of the patients had IL–1β levels which were above the reference range. After the treatment, the number of patients with dyslipidaemia came down, with 23.3% patients having high total cholesterol levels, 43.3% of the patients having elevated triacylglycerol levels, 46.7% patients having low HDL–cholesterol levels and 20% patients having elevated LDL–cholesterol levels. 66.7% of the patients had IL–1β which was above the reference range.

Conclusion: The proportion of dyslipidaemic patients had decreased in the follow up visit, along with a decrease in the disease activity, as were indicated by the decreased levels of IL–1β. The management of dyslipidaemia in RA should be a part of the general cardiovascular risk management. Therefore, a good control of the disease activity should be given priority, so that both the quality of life and the long–term outcomes can be improved.

Key words: Cardiovascular risk, Dylipidaemia, Interleukin-1β, Rheumatoid arthritis

INTRODUCTION

Rheumatoid Arthritis (RA) is one of the common inflammatory diseases of the joints, of unknown aetiology, which is characterized by symmetric erosive synovitis and an extra articular involvement like Rheumatoid nodules, Rheumatoid vasculitis, Pleuropulmonary manifestations, Felty's syndrome, etc [1]. The prevalence of Rheumatoid arthritis is between 0.7% to 1.5%. Malviaya et al., found the prevalence in the Indian rural population to be 0.75% [2].

Three important inflammatory cytokines are implicated in the pathophysiology of Rheumatoid arthritis, i.e., Interleukin (IL)–1β, Interleukin (IL)–6 and the Tumour Necrosis Factor (TNF) - α. Among these, IL–1 is the most potent inflammatory mediator which causes damage to the joints and which causes systemic effects by stimulating the synthesis of IL–6 and the C-reactive protein [3].

RA itself, particularly its chronic inflammatory component, could be an independent cardiovascular risk factor and is associated with accelerated atherosclerosis [4]. Atherosclerotic cardiovascular disease is the major cause of the mortality in Rheumatoid arthritis. Dyslipidaemia is an important risk factor for cardiovascular disease and it is influenced by the disease activity of Rheumatoid arthritis, which is the combined effect of these inflammatory mediators [5].

The pattern of the lipid profile, which has been described in Rheumatoid arthritis, comprises low total cholesterol (TC) levels, low density lipoprotein cholesterol (LDL–C) levels, low high density lipoprotein cholesterol (HDL–C) levels and elevated Triacylglycerol (TG) levels [6].

The data on the inflammatory mediators and the lipid profile in Rheumatoid arthritis patients will help in reducing the morbidity and the mortality.

OBJECTIVES

To study the disease activity in Rheumatoid arthritis patients by measuring the serum levels of interleukin-1β (IL–1β), to find out the proportion of the rheumatoid arthritis patients who suffer from dyslipidaemia, to correlate the disease activity with the lipid profile and to look for the change in the lipid levels and the disease activity after an intervention with antirheumatic therapy.

MATERIALS AND METHODS

This study was conducted over a period of one and half years, from January 2010 to June 2011. It was approved by the ethical committee of the institute and informed consents were obtained from all the subjects who took part in the study.

This study comprised the Rheumatoid arthritis patients who attended the outpatient and inpatient departments of Medicine of Victoria Hospital and the Bowring and Lady Curzon hospitals which are attached to Bangalore Medical College and Research Institute, Bangalore, India.

The patients who fulfilled the modified American College of Rheumatology criteria for Rheumatoid arthritis [7] were included in the study.

The patients with a history of other chronic inflammatory disorders and neoplastic conditions which are known to influence the serum levels of the inflammatory mediators, those with a history of co-morbid conditions which are known to influence the lipid profile, like Diabetes mellitus, cardiac disease, chronic renal failure and hypothyroidism and the patients who were on statins and other lipid lowering drugs, were excluded from the study.

This study consisted of thirty cases of RA, whose serum samples were taken and analysed twice, i.e., before starting the treatment and after 12 weeks of starting the treatment with Disease Modifying Anti Rheumatoid Drugs (DMARDs).

Following the selection of the subjects and after obtaining their informed consents with respect to the proposed study, about 5ml of fasting venous blood sample was collected from them, from the median cubital vein by venepuncture. The serum was separated by centrifugation. The lipid profile parameters, IL–1β and the rheumatoid factor were estimated immediately.

Total cholesterol, Triacylglycerols and High Density Lipoprotein cholesterol were estimated by an enzymatic, colourimetric method [8-10] by using a COBAS integra 400 plus analyser.

I. Estimation of the Total cholesterol in Serum (an Enzymatic, colourimetric method) [8].

Principle: Cholesterol esters are cleaved by the action of cholesterol esterase, to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyses the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide which is formed affects the oxidative coupling of phenol red and 4–aminoantipyrine to form a redquinine–imine dye.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{RCOOH}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{quinine-imine dye} + 4H_2O$$

The colour intensity of the dye which is formed is directly proportional to the concentration of cholesterol. It is determined by measuring the increase in the absorbance at 512 nm.

II. The Estimation of Serum Triglycerides (an Enzymatic, colourimetric method) [9].

Principle: Glycerol which is released from the hydrolysis of triglycerides by lipoprotein lipase, is converted by glycerol kinase into glycerol-3-phosphate, which is oxidised by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide oxidises the phenolic chromogen to a red coloured compound.

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + 3\text{RCOOH}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinas}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3- phosphate} + O_2 \xrightarrow{\text{Glycerol phosphate oxidase}} \text{Dihydroxy acetone Phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4-cholorophenol} \xrightarrow{\text{Peroxidase}} \text{4-(p-benzoquinone-monoimino)-phenazone} + 2\,H_2O + \text{HCl}$$

The intensity of the colour which is developed for triglycerides in the sample, is measured at 510 nm.

III. Estimation of the Serum High Density Lipoprotein-cholesterol (a Homogenous enzymatic, colourimetric method) [10].

Principle: In the presence of magnesium ions and dextran sulfate, water-soluble complexes with LDL, VLDL and the Chylomicrons are formed, which are resistant to the Polyethylene Glycol (PEG)-modified enzymes. The cholesterol concentration of HDL–cholesterol is determined enzymatically by cholesterol esterase and by cholesterol oxidase which is coupled with PEG to the amino groups (approximately 40%). The cholesterol esters are broken down quantitatively into free cholesterol and free fatty acids by cholesterol esterase. In the presence of oxygen, cholesterol is oxidised by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide.

$$\text{HDL–cholesterol esters} + H_2O \xrightarrow{\text{PEG-cholesterol esterase}} \text{HDL–cholesterol} + \text{RCOOH}$$

$$\text{HDL–cholesterol} + O_2 \xrightarrow{\text{PEG-cholesteroloxidas}} \Delta\text{4-cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{HSDA\#} + H^+ + H_2O \xrightarrow{\text{peroxidase}} \text{Purple blue pigment} + 5H_2O$$

#Sodium N (2-hydroxy-3-sulfopropyl)-3, 5-dimethoxyaniline

The colour intensity of the blue quinoneimine dye which is formed is directly proportional to the HDL–cholesterol concentration. It is determined by measuring the increase in the absorbance at 583nm.

The Low Density Lipoprotein cholesterol and the Very Low Density Lipoprotein cholesterol were calculated by using Friedewald's equation [11].

After the estimation of the total cholesterol, triglycerides and HDL cholesterol, the values of LDL–cholesterol and VLDL–cholesterol were calculated.

Calculation

LDL and VLDL–cholesterol were calculated by using Friedewald's equation.

$$\underset{\text{(mg/dl)}}{\text{LDL cholesterol}} = \underset{\text{(mg/dl)}}{\text{Total Cholesterol}} - \underset{\text{(mg/dl)}}{\text{HDL Cholesterol}} - \frac{\text{Triglycerides (mg/dl)}}{5}$$

$$\text{VLDL cholesterol} = \frac{\text{Triglycerides (mg/dl)}}{5}$$

mg/dl

IL–1β was estimated by a chemiluminescence method by using an IMMULITE 1000 analyser [12].

IL–1β estimation was done by a solid phase, two-site chemiluminescent immunometric assay.

First, the alkaline phosphatase conjugate (reagent) was bound to the bead (within the test unit) during the immunological reaction.

The amount of alkaline phosphatase which is captured is directly proportional to the concentration of IL–1 β in the patient sample.

Once the test unit is washed, a luminogenic substrate is added to the test unit and it is moved onto the luminometer chain.

In the luminogenic reaction, the substrate (an adamantyl dioxetane phosphate) is dephosphorylated into an unstable anion intermediate by the alkaline phosphatase conjugate which is captured on the bead. The unstable intermediate emits a photon upon decomposition. The amount of light which is emitted is directly proportional to the amount of bound alkaline phosphatase and in turn, to the amount of IL–1β in the sample.

Statistical Methods: A descriptive statistical analysis was carried out in the present study. The results of the continuous measurements were presented as Mean ± SD (Min to Max) and the results of the categorical measurements were presented as Number (%). The significance was assessed at a 5 % level of significance.

The Student's "t" test (two tailed, independent) was used to find the significance of the study parameters on a continuous scale between the two groups (an inter group analysis on the metric parameters).

Statistical Software: The Statistical softwares, namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 ,Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel were used to generate graphs, tables, etc.

RESULTS AND OBSERVATIONS

As have been shown in [Table/Fig- 1] and [Table/Fig- 2], the mean level of IL–1β before the treatment was found to be 21.98 pg/ml, with an SD of 2.73 pg/ml and after the treatment, the mean was found to be 9.22 pg/ml, with an SD of 2.14 pg/ml. There was a statistically significant decrease in the IL–1β levels after the treatment.

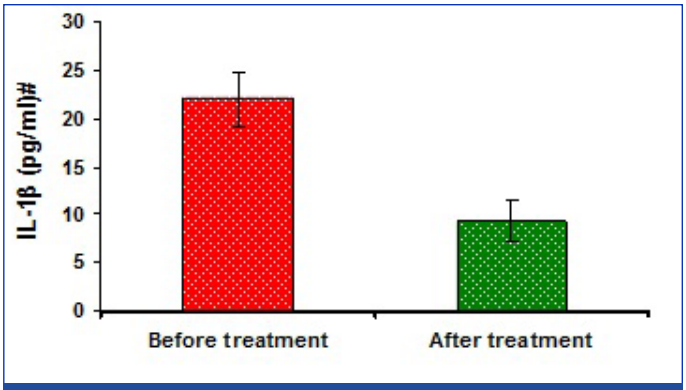
As have been described in [Table/Fig- 3], before the treatment, 4 (13.3%) patients had IL–1β levels of <5 pg/ml and 26 (86.7%) patients had IL–1β levels of >5 pg/ml. After the treatment, 10 (33.3%) patients had IL–1β levels of <5 pg/ml and 20 (66.7%) patients had IL–1β levels of >5 pg/ml.

As have been shown in [Table/Fig- 4], the mean total cholesterol level before the treatment was 185.53mg/dl with an SD of 43.70 mg/dl and after the treatment, the mean was 174.13 mg/dl with an SD of 34.86 mg/dl. The mean of triacylglycerol before the treatment was 173.13 mg/dl with an SD of 92.94 mg/dl and after the treatment, the mean was 147.93 mg/dl with an SD of 57.47 mg/dl. There was a statistically significant decrease in the triacylglycerol levels after the treatment. The mean of HDL–cholesterol before the treatment was 36.33 mg/dl with an SD of 7.04 mg/dl and after the treatment, the mean was 40.20 mg/dl with an SD of 8.89 mg/dl. There was a statistically significant increase in the HDL–cholesterol levels after the treatment. The mean of LDL–cholesterol before the treatment was 114.12 mg/dl with an SD of 35.94 mg/dl and after the treatment, the mean was 107.03 mg/dl with an SD of 31.16 mg/dl.

As has been shown in [Table/Fig- 5] and [Table/Fig- 6], there was no significant correlation between IL–1β and the lipid parameters.

	Before treatment (n=30)	After treatment (n=30)	p value	Effect size
IL–1β (pg/ml)#	21.98±2.73 (4.0-158.0)	9.22±2.14 (4.0-56.90)	0.003**	0.90(L)

[Table/Fig- 1]: Evaluation of IL–1β before and after treatment
L: Large; # Minimum value of IL–1β (pg/ml) considered in the analysis is 4 (pg/ml) for <5 (pg/ml), IL–1β is log transformed and presented in original units.** Strongly significant (p value: < 0.01).



[Table/Fig- 2]: Evaluation of IL–1β before and after treatment

Parameters	Before treatment (n=30)		After treatment (n=30)	
	No	%	N0	%
IL–1β (pg/ml)				
<5.0	4	13.3	10	33.3
>5.0	26	86.7	20	66.7

[Table/Fig- 3]: Levels of IL–1β of patients studied

Lipid parameters	Before treatment (n=30)	After treatment (n=30)	p value	Effect size
Total cholesterol (mg/dl)	185.53±43.70 (99-292)	174.13±34.86 (106-259)	0.029*	0.29(S)
Triacylglycerol (mg/dl)	173.13±92.94 (55-574)	147.93±57.47 (41-285)	0.060+	0.34(S)
HDL–Cholesterol (mg/dl)	36.33±7.04 (26-54)	40.20±8.89 (24-58)	0.021*	0.50(M)
LDL–Cholesterol (mg/dl)	114.12±35.94 (53.6-211.30)	107.03±31.16 (54.5-178.0)	0.254	0.21(S)
VLDL–cholesterol (mg/dl)	34.78±18.56 (11.10-115.0)	29.59±11.49 (8.30-57.00)	0.053+	0.35(S)

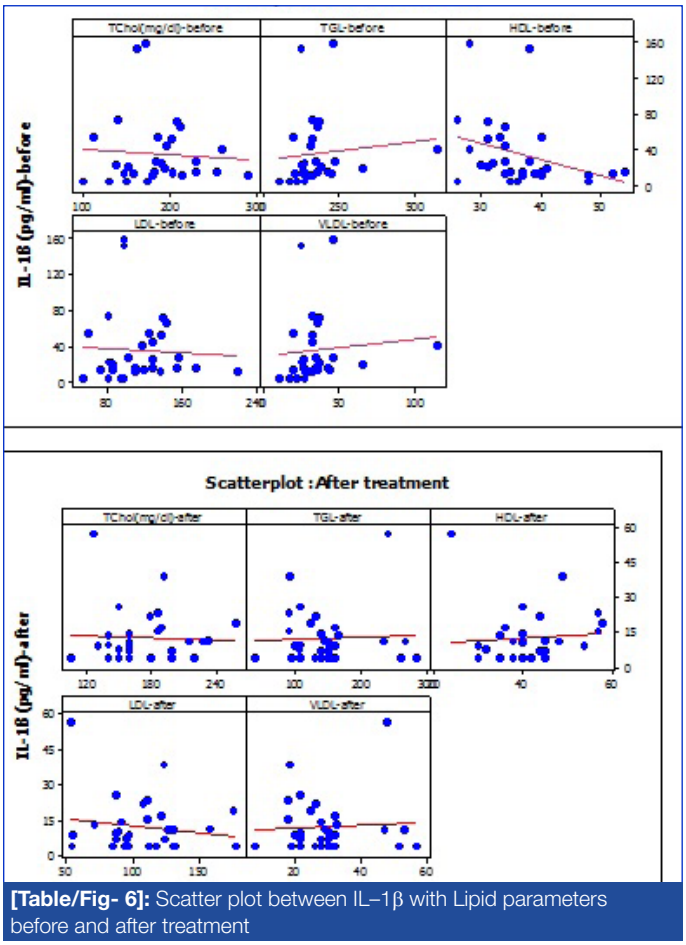
[Table/Fig- 4]: Evaluation of lipid profile parameters before and after treatment S: Small; M: Moderate.
+ Suggestive significance (p value: 0.05 to < 0.10)
* Moderately significant (p value: 0.01 to < 0.05) Results are presented as Mean ± SD (Min-Max), p value obtained by student't' test (Paired).

DISCUSSION

Rheumatoid arthritis, an immune-inflammatory condition, aggravates the metabolic syndrome (which includes dyslipidaemia). In RA, although the primary site of inflammation is the synovial tissue, cytokines like the Tumour Necrosis Factor (TNF)-α, IL–1β and IL–6 are also released into the systemic circulation and this leads to dyslipidaemia [1]. These circulating cytokines alter the function of the distant tissues, which include the adipose tissue, the skeletal muscle, liver, etc, which in turn, leads to dyslipidaemia. There is increased Free Fatty Acid (FFA) release in the adipose tissue, increased FFA and TG synthesis in the liver and a reduced lipoprotein lipase activity. Lipoprotein lipase is the principle catabolic enzyme for the TG-rich lipids. High TG levels reduce the HDL–C by virtue of the neutral lipid exchange and this same

Numbers	Before treatment (n=30)		After treatment (n=30)	
	r value	p value	r value	p value
IL–1β (pg/ml)vs Total cholesterol (mg/dl)	-0.069	0.717	-0.045	0.812
IL–1β (pg/ml)vs Triglycerides (mg/dl)	0.101	0.596	0.049	0.068
IL–1β (pg/ml)vs HDL–Cholesterol (mg/dl)	-0.335	0.071+	0.068	0.722
IL–1β (pg/ml)vs LDL–Cholesterol (mg/dl)	-0.062	0.746	-0.148	0.435

[Table/Fig- 5]: Pearson correlation between IL–1β with lipid profile parameters



[Table/Fig- 6]: Scatter plot between IL–1β with Lipid parameters before and after treatment

process promotes the synthesis of the small dense LDL [5].

The RA patients appeared to have a high prevalence of an abnormal blood lipid profile, as was supported by three Indian studies which were conducted by Hadda V et al., [6], Grover S et al., [13] and Vottery R et al., [14] with 96, 56 and 25 patients respectively. Vottery R et al.,'s study showed lower lipid levels and a negative correlation with the disease activity. Grover S et al.,'s study demonstrated only raised total cholesterol levels. Hadda V et al.,'s study showed that 38.5% of the patients were dyslipidaemic, the commonest being low HDL–cholesterol in 34.3% of the patients and a trend was observed towards the normalisation of the lipids and a decrease in the disease activity in the follow up visits.

The present study showed that before the treatment, 36.7% of the patients had high total cholesterol levels, 53.3% of the patients had high triacylglycerol levels, 73.3% of the patients had decreased HDL–cholesterol levels and that 33.3% of the patients had high LDL–cholesterol levels. 86.7% of the patients had IL–1β which was >5 pg/ml.After the treatment, the number of patients with dyslipidaemia came down, with 23.3% of the patients having high total cholesterol levels, 43.3% of the patients having elevated triacylglycerol levels, 46.7% of the patients having low HDL–cholesterol levels and 20% of the patients having elevated LDL–cholesterol levels. 63.7% of the patients had IL–1β which was above the reference range. Thus, the proportion of the dyslipidaemic patients had decreased in the follow up visit, along with a decrease in the disease activity, as was indicated by the decreased levels of IL–1β.

In general, the antirheumatic treatment has moderate effects on the lipid profile. Therefore, it is unlikely that the observed beneficial effects of the antirheumatic drug treatment on the cardiovascular morbidity and mortality in RA is mediated through its effects on the lipid metabolism. They (the DMARDs) act by reducing the monocyte mediated secretions of IL–1 and the tumour necrosis factor, thus controlling the effects of these mediators on the lipid metabolism in the adipose tissue and the liver. Whilst the routine use of statins as a disease–modifying therapy for the patients with RA is not yet a routine practice, their use in selected patients with abnormal lipid profiles could also benefit their arthritis, as was also supported by White et al., [15]. The management of dyslipidaemia in RA should be a part of the general cardiovascular risk management, as was also supported by Nurmohamed et al., [16]. These data raise the possibility of the addition of an improved method of primary–prevention interventions, such as the use of the HMG–CoA (3–hydroxy–3methylglutaryl coenzyme A) reductase inhibitors.Therefore, a good control of the disease activity should be the priority, given that both the quality of life and the long-term outcomes can be improved. The limitations of our study were the small sample size and that only one follow-up visit was included. Because of only one follow–up, we cannot comment on the long term effects of the control of the disease activity on lipids. While screening for dyslipidaemia seems to be warranted in all the patients, the interventional strategies need further study.

CONCLUSIONS

Our study reveals that lipid abnormalities are common in the patients who suffer from rheumatoid arthritis, with low HDL–cholesterol being the commonest dyslipidaemia which is being encountered. The next common abnormality is elevated triacylglycerol levels. The disease activity was also high, as was indicated by the significantly elevated levels of IL–1β.

The proportion of dyslipidaemic patients had decreased in the follow up visit, along with a decrease in the disease activity, as was indicated by decreased levels of IL–1β. The management of dyslipidaemia in RA should be a part of the general cardiovascular risk management. Therefore, a good control of the disease activity should be the priority, given that both the quality of life and the long-term outcomes can be improved.

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