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Study of lipid profile in pulmonary tuberculosis patients and relapse cases in relation with disease severity - A pilot study

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Abstract

Background: Malnutrition and Tuberculosis are synergistically associated to each other. Any inflammatory condition following infection causes release of free radicals and reactive oxygen species (ROS) which adversely affects host lipid by causing enhanced lipid peroxidation.

Objective: To determine the level of Lipid fractions in Newly Diagnosed and Relapse Pulmonary Tuberculosis Patients and also to find correlation between serum lipid level with inflammation and disease severity.

Materials and methods: 32 newly diagnosed and 26 relapsed cases to PTB were recruited for the study. Patients were both male and female with average age 37.16±1.2 years and 39.44±1.5 years respectively. 25 age and gender matched healthy subjects that were non family members of patients were taken as controls for comparison. Fasting serum lipid profile (Total Cholesterol (TC),Triglyceride(TG), HDL-cholesterol (HDL-c) , Low density Lipoprotein (LDL) and Very Low density Lipoprotein (VLDL) and CRP along with ADA were estimated**.**

Results and discussion: All lipid parameters were significantly ($p<0.05$) low in both newly diagnosed and relapse cases of Pulmonary Tuberculosis (PTB) than controls. TC and LDL level were significantly higher in relapsed patients than new PTB cases. Inflammatory markers (ADA and CRP) increased significantly ($p<0.05$) in both new and relapsed group according to control group. Cholesterol and LDL are moderately correlated to serum ADA as compared to CRP, however no significant correlation was observed between other lipid parameters with ADA or CRP. However, lipid parameters are well correlated with smear positivity extent indicating that SPE is a better measure to assess disease severity which involves progressive decrease in serum lipids**.**

Conclusion: Hypocholesterolemia exists in both newly diagnosed and relapse PTB patients and is one of the many nutritional factors predisposing to TB infection. Serum lipids affect overall strength of immune system with cholesterol being most widely studied in this aspect. SPE (Smear Positivity Extent) shows strong correlation with serum lipids in PTB patients , indicating its reliability in assessing dyslipidemia in PTB patients.

Keywords: Pulmonary Tuberculosis, Lipid Profile, Cholesterol

Abbreviations: PTB - Pulmonary Tuberculosis, ADA - Adenosine deaminase, SPE - Smear Positivity Extent, ROS - Reactive oxygen species, MTB - Mycobacterium Tuberculosis, AFB - Acid Fast Bacilli

Introduction

Tuberculosis (TB) is a potentially fatal contagious disease and remains a major global health care issue(1). It is caused by a bacterial microorganism, the tubercle bacillus or *Mycobacterium tuberculosis* (MTB). It can affect any part of the body but is mainly a lung disease where it forms localized infection after inhalation. Almost one third of the world's population is infected with *Mycobacterium tuberculosis* and the majority of these individuals live in less developed countries *(WHO, Global Tuberculosis Control, 2011*). Worldwide, TB is responsible for more than 1.5 million deaths every year. Almost 40 percent of the population in India is infected with the TB pathogen, with every fifth new patient worldwide living in this sub-continent. It is the 2nd leading cause of death killing 2 million people each year(2). MTB usually affects apices of lungs. Infection with bacilli does not necessarily lead to active disease as the immune response of most individuals can successfully contain, but not eliminate the infection. A decline in immune response due to poor nutritional status can lead to active infection(3). It is characterized by general symptoms such as unexplained cough, dehydration/vomiting, unexplained tiredness, loss of weight, high remittent or intermittent pyrexia loss of appetite, severe cough sometimes with blood in the sputum, exhaustion and night sweats(4). For the diagnosis of tuberculosis, microbiologic, genetic, immunogenic and biochemical methods are used. One of the biochemical methods is the measurement of ADA (adenosine deaminase) activity(5). ADA converts adenosine into inosine and deoxyadenosine into deoxyinosine. It is an indicator of active cellular immunity by its role in proliferation and differentiation of T-lymphocytes(6). Active TB is an acute inflammatory condition associated with tissue injury due to increased generation of free radicals and ROS (Reactive oxygen species). ROS and RNI (Reactive Nitrogen Intermediates) are produced as a consequence of phagocytic respiratory burst(7). Further, the disease process of TB involves cellular immunity and phagocytosis of MTB by macrophages and releases of interferons, TNF-αand other cytotoxic molecules. Phagocytic activity of macrophages, neutrophils and monocyted also generates ROS and free radicals that not only have destructive effect on serum lipids (by lipid peroxidation) but also contributes to immune suppression. Extent of inflammation determines the severity of disease as indicated by ESR and acute phase protein C-reactive protein (CRP). The physiological roles of CRP are numerous, one of the critical functions being its importance in host defense. C-reactive protein is an acute phase protein that is established marker of acute inflammation and its serum concentration is determined to assess the grade of systemic inflammation(8).

Association between TB and malnutrition is well recognized. TB can lead to malnutrition and malnutrition may predispose to TB. Lipids are important constituents that determine nutritional status and at the same time participate in immune function(9). Role of Cholesterol and other serum lipoproteins is well known in CVD. However, increasing evidence indicates link between low Cholesterol level and a number of human diseases including TB(10). Despite existence of such link it is not known to which extent hypocholesterolemia predisposes to MTB infection and whether ongoing treatment affects lipid parameters.

With this background the study was conducted to estimate lipid fraction (Cholesterol, Triglyceride, HDL-cholesterol, LDL and VLDL) in PTB patients and to find out whether any difference occurs in lipid levels of newly diagnosed Pulmonary Tuberculosis Patients and relapse cases (who are on ATT previously). We also aimed to find association of lipid parameters with disease severity (assessed by sputum

status) and inflammation as indicated by the level of serum ADA and Acute Phase Protein such as CRP.

Materials and methods

The study was conducted in the Institute of Respiratory Diseases (IRD) in association with Department of Biochemistry, SMS Medical College, Jaipur. 58 Adult patients (both male and female) from low socio-economic status diagnosed with Pulmonary Tuberculosis (PTB) of which 32(M=24;F=8) were newly diagnosed and 26 (M=23;F=3) relapse cases were recruited for the study. 25 (M=18; F=7) healthy, age matched individuals tested free of MTB without any previous or present symptoms of Tuberculosis or any other pulmonary disease and non family member of patients served as Controls. Following diagnostic criteria was used for PTB 1. Positive culture for MTB. 2. Positive (2 consecutive samples) smear for AFB. 3. Typical chest X-ray showing bilateral upper zone involvement with or without cavitations & with/without +ve sputum smear but with typical PT symptoms. 4. Patients with 2 or 3 criteria had to show clinical and radiological improvements with anti tuberculosis therapy. Relapse cases are patients who have completed treatment and declared cured, however they came up with typical PTB symptoms with +AFB smear or radiological detoriation.

Patients suffering from drug resistant TB (MDR), extrapulmonary TB, those with significant renal, cardiac, neoplasm or respiratory disease (other than PTB like lung cancer) etc., diabetes, endocrine or genetic disorder were excluded from the study. HIV positive cases, Pregnant or lactating women were also excluded. All subjects gave their written consent to participate in the study.

Sample collection and bacteriological examination

After an overnight fast (12 hrs), venous blood was drawn from anticubital vein of each subject by using aseptic technique in plain and EDTA vials. Two consecutive sputum samples were collected and subjected to Acid fast Staining. In order to determine Smear Positivity index, number of Acid Fast Bacilli (AFB) were counted and analysed as follows:1. No AFB in 100 fields-negative; 1-9 AFB in 100 fields-scanty ; 10-99 AFB in 100 fields- +1 ; 1-10 AFB per field-+2 and more than 10 AFB per field- +3.

Measurement of biochemical parameters

Total Cholesterol (TC) (cholesterol oxidase-peroxidase, Accurex) , Triglyceride(TG) (Glycerol Phosphate oxidase, Accurex) , HDL-cholesterol (HDL-c) (Direct Homogenous method, Accurex) were estimated by enzymatic method. Low density Lipoprotein (LDL) and Very Low density Lipoprotein (VLDL) were calculated by using Friedwald formula. ADA was estimated by enzymatic method using Diazyme assay kit, CRP was estimated by Turbidimetric method (Accurex) and ESR by direct Vesmatic Cube ESR Analyzer.

Statistical analysis

Quantitative data were expressed as mean±SD. Comparison was made using student-t test (independent sample t-test.). P value less than 0.05 was considered significant. Correlation between various parameters was studied by Pearson Correlation.

Results and discussion

The study involves 32 newly diagnosed PTB patients (group 1), 26 relapse cases (group 2) and 25 controls (group 3). Average age of Group 1 patient was 37.16± 1.2 years and included 24 males (75.0 %) and 8 females (25%). Average age of Group 2 patient was 39.44±1.5 years and include 23 males (88.5%) and 3 females (11.5%). Average age of Group 3 subjects was 32.8±6.1 years and includes 18 (72.0%) males and 7(28%) females. There was no significant difference in age ($p<0.05$) between all three groups. Newly diagnosed group consist of 25% sputum negative, 50% cases +1, 12.5%

cases +2and 12.5 % cases +3. Relapse group consist of 19.2% negative, 46.2% cases +1, 19.2% cases +2 and 15.4% cases of +3 sputum positivity. All controls were sputum negative. General characteristics of all subjects are summarized in Table 1.

Table 1: General characteristics of all subjects

Characteristics	Group 1 (newly Diagnosed)	Group 2 (relapsed)	Group 3 (controls)
Number of Subjects	32	26	25
Males (N)	27 (75.0)	23 (88.5)	18 (72)
Females(N)	8 (25.0)	3 (11.5)	7 (28)
Average Age (mean±SD) in years	37.16± 1.2	39.44±1.5	32.8±6.1
SPUTUM STATUS			
Negative	8(25)	5(19.2)	25(100)
+1	16(50)	12 (46.2)	0(0)
+2	4(12.5)	5(19.2)	0(0)
+3	4(12.5)	4(15.4)	0(0)

Values are numbers and values in parenthesis are percent.

Table 2: Inflammatory parameter of subjects

Sr. No.	Inflammatory Parameters	Group 1 (new)	Group 2 (relapsed)	Group 3 (control)	Significance		
					I vs III	II vs III	I vs II
1.	ADA (U/L)	22.9±0.25	18.6 ± 0.7	6.27 ± 2.6	S	S	S
2.	CRP (mg/ml)	48.75±23.7	32.2 ± 12.5	1.84 ±1.5	S	S	S
3.	ESR (mm)	51.16± 2.5	46.48±6.0	19.3 ± 6.2	S	S	NS

Values are mean±SD; S - Significant; NS - Non Significant

In the present study, newly diagnosed and relapsed cases show significantly higher values of CRP and ESR than controls ($p<0.05$) (table 2) (Fig. 1). Similarly, mean ADA of Newly diagnosed group was 22.9±0.25 and that of relapsed group was 18.6±0.7 IU/L were significantly higher than control 6.27±2.6 (table 2) (Fig 1). When group 1 and group 2 were compared, newly diagnosed patients show significantly ($p<0.05$) higher level of these inflammatory parameters. In MTB infection, immune response is initiated with macrophages and neutrophils phagocytosed the bacteria at the site of infection accompanied with the production of pro-inflammatory cytokines TNF-α and IL-6 that induces fever and hepatic synthesis of acute phase proteins. Extent of inflammation determines disease severity and is indicated by parameters like ESR, CRP and ADA. CRP is a generalized marker of chronic inflammation. It reflects the extent of oxidative stress and indicates disease severity and mortality risk. Active TB is associated with significant rise in CRP due to increased levels of cytokines(11). A raised CRP and ESR level are useful in differential diagnosis of TB patients with sputum negative AFB(12). ESR is regarded as generalized marker of any inflammatory condition and is used in differential diagnosis of tuberculosis and pneumonia.

Various authors have shown rise in ESR in PTB patients with some showing direct linkage of ESR with radiological stage of disease(12). ADA is a significant indicator of active cellular immunity and is widely distributed in human tissue specially in T-Lymphocytes. Stimulated alveolar macrophages and monocytes produces local inflammatory response causing a significant rise in serum ADA level. ADA is found to be increased in patients with tubercular effusion(13). ADA assay has been suggested as an inexpensive and sensitive marker for routine screening of PTB with a cut-off 33.3 IU/L(14). After ATT initiation, both leukocytes and lymphocytes population goes down that parallels serum ADA and is responsible for significantly lower values in relapse group than newly diagnosed cases as found in our study. Studies have also shown high ADA at the time of diagnosis of TB that decreases after two months of treatment and that serum ADA also depends on treatment effectiveness(15).

Table 3 shows the level of Lipid parameters in all subjects. Both patient group (Newly diagnosed and relapse) have low total Cholesterol i.e. 114.4±15.5 and 123.4±5.5 respectively than control group with TC level of 200.37±34.7 (Fig 2). Significant difference exsist between Group1 and Group 2 patients in their TC level ($p<0.05$). Nutrition is the most important factor that affects susceptibility to any infection(16). Association between TB and malnutrition is well recognized. TB can lead to malnutrition and malnutrition may predispose to TB(17). It is estimated that one third of worlds' population is infected with MTB, yet only a small proportion develops active disease. Therefore, some specific condition must predispose these individuals to develop active TB disease(18). Lipids are essential factors that determine our nutritional status. Low lipid level leads to increased susceptibility to various infection like TB(19,20). Cholesterol is the most studied lipid in this direction. It constitutes 30% of lipid content of Plasma membrane and affects its fluidity (21).

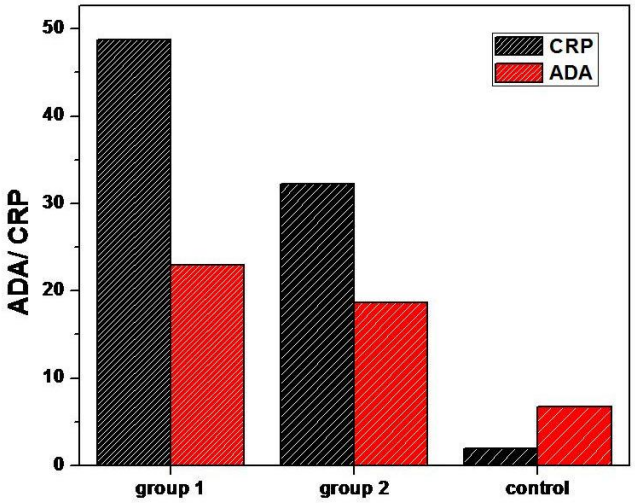

Fig 1: level of Inflammatory Markers in Newly Diagnosed, Relapse and control subjects.

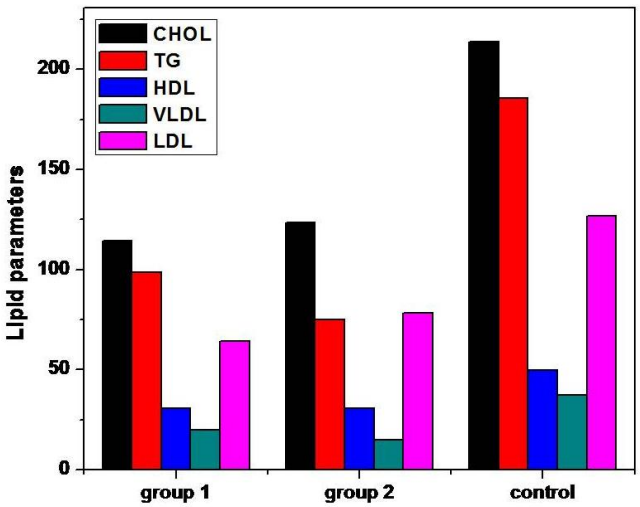

Fig 2: Lipid parameters in Newly Diagnosed, Relapse and Control subjects.

Secretory process of phagocytic cells like Macrophages requires cholesterol (such as cell motility, exocytosis and endocytosis). Their phagocytic activity was found to be deranged in cholesterol deficiency(22). It is also required for bacterial entry into host macrophages and for growth and multiplication in host. MTB prefertially uses fatty acids than carbohydrates as primary

carbon source during chronic infection(23). Around 250 genes potentially involved in lipid metabolism have been discovered in MTB(24). These observations indicate utilization of Cholesterol by MTB and hence host cellular pool should decrease after MTB infection.

Cholesterol is an important nutritional factor that affects overall strength of immune system. High cholesterol have beneficial effect on immune system because hypocholesterolemic men had significantly lower circulating lymphocytes , total T-cells, helper T- cells and CD8+ cells than hypercholesterolemic men(25). Further, incidence of anti-tuberculous drug ressistance decreases with high blood cholesterol(26). These facts proposes hypocholesterolemia as a risk factor for PTB. Malnutrition and low socio-economic status of our study population are the key factors that make them more prone to TB infection. Impaired rate of lipid production and enhanced rate of lipid catabolism during chronic stage of disease is also responsible for reduced level of cholesterol in PTB cases. When both Newly diagnosed and relapsed cases were considered together, serum cholesterol show moderately negative correlation with SPE and poor correlation with ADA and CRP (table 4) (Fig 3a.). CRP is a generalized marker of chronic inflammation and ADA is related to cellular immunity. Thus, SPE is a better indicator of disease severity.

TABLE 3: Lipid profile of subjects

Sr. No.	Biochemical Parameter	Group 1 (Newly diagnosed)	Group 2 (Relapsed)	Group 3 (Control)	Significance		
					I vs III	II vs III	I vs II
1.	Total Cholesterol (mg %)	114.41±15.5	123.48±5.5	200.37±34.7	S	S	S
2.	Triglyceride (mg %)	98.6±22.5	74.9±10.0	174.12±24.2	S	S	S
3.	HDL-chol (mg %)	30.37±2.0	30.41±7.7	46.48±4.67	S	S	NS
4.	LDL (mg %)	64.31±13.0	78.08±15.2	119.06±34.6	S	S	NS
5.	VLDL (mg %)	19.72±4.5	14.9±2.0	34.82±4.82	S	S	S

Values are mean±SD; S - Significant; NS - Non Significant

TABLE 4: Correlation between Lipid parameters and inflammatory markers

Sr. No.	Lipid parameters	ADA (Correlation Coefficient=r)	CRP (Correlation Coefficient =r)	SPE (Correlation Coefficient =r)
1.	Total Cholesterol	-0.32	-0.20	-0.56
2.	Triglyceride	-0.12	-0.12	-0.58
3.	HDL-chol	-0.10	-0.05	-0.66
4	LDL	-0.35	-0.20	-0.32
5	VLDL	-0.11	-0.12	-0.50

Fig 3: Correlation between Smear Positivity Extent (SPE) and Lipid parameters 3(a) with Cholesterol, 3(b) with TG, 3(c) with HDL, 3(d) with VLDL and 3(e) with LDL.

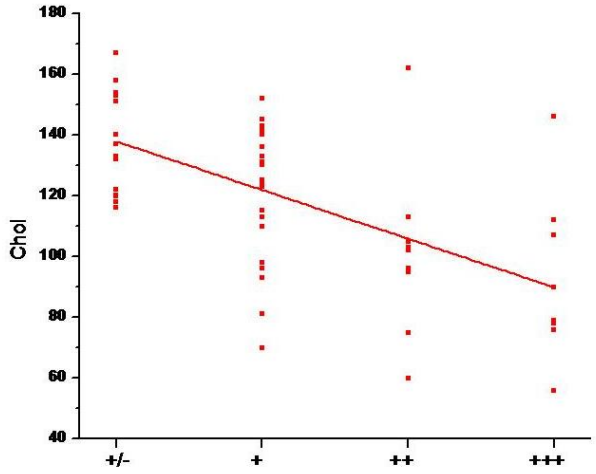

Fig. 3a

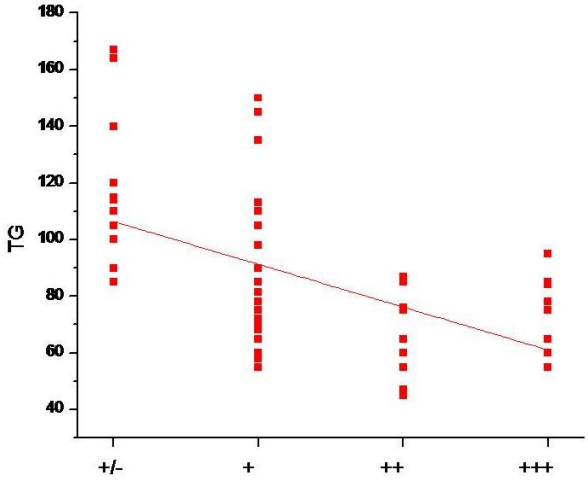

Fig. 3b

In our study, TG and LDL level were also significantly ($p<0.05$) lower in PTB cases (Newly diagnosed and relapse) than controls (table 3) (Fig 2). PTB is associated with increased generation of free radicals due to oxidative stress arising as a result of enhanced phagocytic activity of monocytes and macrophages. TG and LDL are major components of cell membrane that are rapidly attacked by ROS and free radicals. Membrane bound TG and LDL are catabolised by lipid peroxidation.

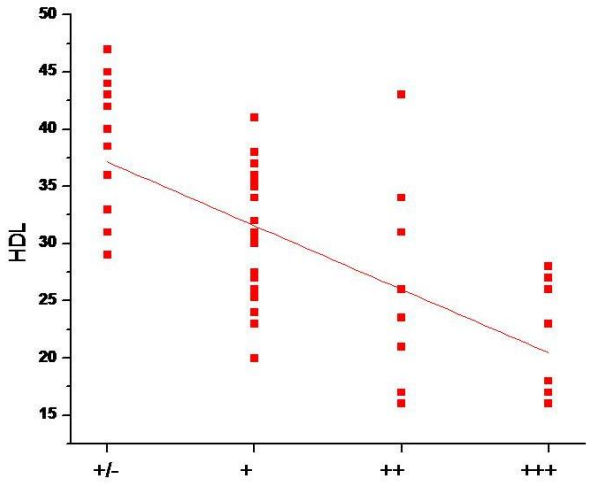

Fig. 3c

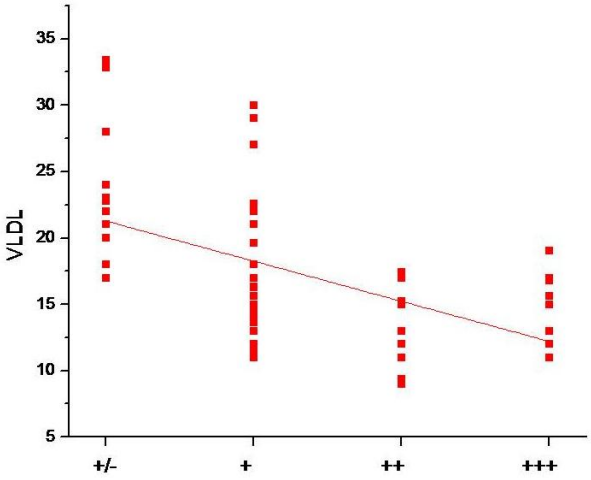

Fig. 3d

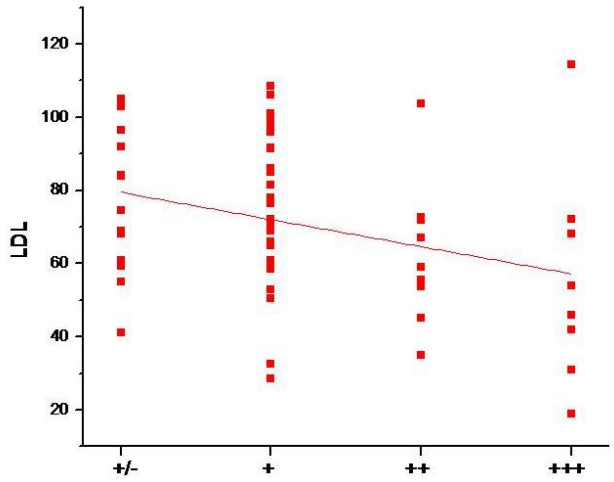

Fig. 3e

Serum lipid may therefore be used to replenish membrane bound TG and LDL causing a decrease in their level in PTB

cases as found in our study. Free radicals also play role in pathogenesis of TB causing lung fibrosis. High level of lipid peroxidation(27) and low level of antioxidant concentration is seen in all categories of PTB cases(28). TG and LDL are also found to be more correlated to SPE than to ADA and CRP (table 4), though correlation was only low to moderate (Fig. 3.b and e). This may be due to various dietary and metabolic factors influence serum TG and LDL level.

In our study, mean HDL-cholesterol in newly diagnosed and relapsed cases were 30.37±2.0 and 30.41±7.7 mg/dl respectively and was significantly lower than control group (46.48±4.67) (table 3) (Fig. 2). HDL-cholesterol protects arterial wall of circulatory system(29). HDL-cholesterol is influenced by body's metabolic state and complete derangement in lipid production and increased catabolism (via lipid peroxidation)results in low HDL-cholesterol in our study population. HDL-cholesterol shows poor correlation with ADA and CRP but strong correlation with SPE(fig. 3c). The fact that HDL normalizes after anti-tubercular treatment(30) and that a cholesterol rich diet accelerates bacteriologic sterilization of sputum in PTB cases(31) support that dyslipidemia is observed in PTB and is associated to disease severity. HDL-cholesterol catabolism increases during inflammation. Response to inflammation during acute phase of TB is characterized by an over expression of proteins such as phospholipase A2 and circulating amyloid A which further stimulates HDL-C catabolism(32). The study suggest direct and essential role of lipids in immunity in PTB and proposes lipid supplementation in order to prevent occurrence of relapse and improve nutritional status of vulnerable group.

Conclusion

In conclusion, we found that dyslipidemia exists in both newly diagnosed and relapsed PTB patients. This dyslipidemia along with hypocholesterolemia does not show immediate improvement with anti TB therapy as found in relapsed cases. This suggest important role of Lipids in immune function. Lipid supplementation can be considered in TB management to prevent drug resistance and occurrence of relapse. Lipid levels shows strong correlation with SPE and no significant correlation with biochemical markers such as ADA and CRP. SPE is thus a more reliable indicator of dyslipidemia in PTB patients. However, drawback here lies in very few numbers of cases with +2 or +3 sputum status recruited under study. Further studies are required with large population to provide additional support to this assertion.

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