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Original Research Article

Study of plasma Leukotriene B4 levels in patients with alcoholic and nonalcoholic fatty liver disease

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Abstract

Background: In worldwide, alcoholic and nonalcoholic fatty liver disease is a most common global health problem. Liver disease is characterized by inflammation and fibrosis. Leukotrienes B4 are implicated in an inflammation of liver cells. The aim of this study was estimation of LTB4 in patient with alcoholic and non alcoholic fatty liver disease.
Materials and Methods: In the present research study, we recruited total (n = 435) subjects and divided in alcoholic fatty liver disease (n = 149); nonalcoholic fatty liver disease (n = 137), and healthy control subjects (n = 150). Plasma LTB4 was measurement of ELISA method.
Results: The present study shown that significantly increased levels of plasma LTB4 ($p<0.001$) in AFLD patients as compared with healthy controls, and also significantly increased ($p <0.001$) in NFALD patients when they compared with healthy controls.
Conclusion: The identification of mechanism underlying the function of receptor that mediates responses of LTB4, and development of novel therapeutic agents for liver disease.
Keywords: Nonalcoholic fatty liver disease (NAFLD), Alcoholic fatty liver disease (AFLD), leukotrine B4 (LTB4).

1. Introduction

Alcoholic fatty liver disease (AFLD) is manifested by alcohol overconsumption [1]. Excessive amount of alcohol results, liver damage, and can be causes inflammation of liver, apoptosis and finally fibrosis of liver cells [2]. Prevalence rate around 25-40% of the alcoholic liver disease is in the general Indian population [3]. NAFLD is excessive accumulation of fats occurs in liver [4], and associated with obesity and metabolic syndrome [5]. NAFLD enhances the progression of fibrosis, liver cirrhosis, and finally liver failure [6]. The prevalence rate of NAFLD is around 9-32% in Indian general population, and with a higher incidence rate with patients of diabetic and obesity patients.

The leukotrines (LTs) are ecosanoids are important for the pathogenesis of asthma, inflammatory disease, and inflammation of liver cells [7-9]. The leukotrienes are containing three conjugated double bonds and first described by Swedish biochemist Bengt Samuelsson (1979) in leukocytes cells but also found in immune cells. Enhanced LTB4 formation by peripheral blood mononuclear cells in liver disease [10]. Very few studies have reported the role of LTB4 in liver diseases. Therefore LTB4 estimation was conducted in AFLD and NAFLD.

2. Material and Methods

The present research works was carried out in the Department of Biochemistry, Sri Aurobindo Institute of Medical sciences (SAIMS) and P.G. Institute, Indore, M.P., India, during Sep 2016 to Aug 2017. Total (n = 435) patients were enrolled from Department of Medicine, Gastroenterology, SAIMS College and Hospital, Indore for the study. 149 (88 males and 61 females) patients having alcoholic fatty liver disease, and 137 (57 males and 78 females) patients were having nonalcoholic fatty liver disease. The age between 30 to 70 years both gender, and 150 were (92 males and 58 females) normal healthy

controls without any complication. Thyroid disease hypertension, pregnancy, cardiac associated liver disease, cancer, asthma, and other infectious diseases were excluded from the study. The study was approved by the Institutional Ethical Committee and patients were recruited for the study after taking their written informed consent. A detailed physical examination was done which included measuring of height, weight and blood pressure.

2.1 Sample collection and analysis of plasma LTB4 and others biochemical parameters

The overnight fasting blood was collected in anticoagulant coated tubes. Blood samples transferred in EDTA tubes were centrifuged at 3000 rpm for 15 min; separate the plasma fractions were stored at -20°C, and estimation of LTB4 level. Estimation of other biochemical parameter was semi auto-analyzer (Erba).

2.2 Statistical analysis

The statistical analysis was carried out by the SPSS statistics version 20.0. Values are presented as means ± standard deviation (Means ±SD). P < 0.05 was considered as significant level.

3. Results

3.1 Age distribution in study groups

The age distribution in alcoholic and nonalcoholic fatty liver disease and healthy control subjects are listed in Table 1. Out of total (n = 435) subjects, 149 AFLD patients 137 were nonalcoholic fatty liver disease. According to age distribution, 61 to 70 years age group subjects were in higher numbers as compare to other age groups.

Table 1: Age distribution of study groups and healthy controls

Age (years)	AFLD (n=149)	NAFLD (n=137)	Controls (n=150)
30-45	33	32	39
46-60	47	46	57
61-70	69	59	54
Total	**149**	**137**	**150**

3.2 Physiological characteristic of AFLD compared to controls

The duration of disease, height, weight and basal metabolic rate (BMI), in AFLD and healthy control are shown in Table 2.

Table 2: Physiological characteristic of AFLD

Parameters	AFLD (n=149)	Controls (n=150)	P value
Height (cm)	172.7±0.11	168±0.09	p<0.05
Weight (kg)	57.03±10.2	58.00±5.32	p<0.30
BMI (kg/m^2)	20.62±2.36	19.56±1.18	p<0.001

Data are presented as mean ± SD, p< 0.05 was considered as significant level.

3.3 Physiological characteristic of NAFLD compared to controls

The duration of disease, height, weight and basal metabolic rate (BMI), in NAFLD and healthy control are shown in Table 3. The Duration of disease, weight and BMI were found significantly increased in NAFLD as compared to healthy controls.

Table 3: Physiological characteristic of NAFLD

Parameters	NAFLD (n=137)	Controls (n=150)	P value
Height (cm)	172.6±0.09	168±0.09	p<0.001
Weight (kg)	138.81±18.41	58.00±5.32	p<0.001
BMI (kg/m^2)	51.04±10.04	19.56±1.18	p<0.001

Data are presented as mean ± SD, p <0.05 was considered as significant level.

3.4 Blood sugar in AFLD compared to controls

The blood sugar in AFLD patients and healthy control are shown in Table 4. The blood sugar found not significantly in AFLD.

Table 4: Fasting blood sugar in AFLD in compared to controls

Parameters	AFLD (n=149)	Controls (n=150)	P value
Blood Sugar (mg)	105.88±30.83	99.93±20.93	p<0.051

Data are presented as mean ± SD, p <0.05 was considered as significant level.

3.5 Blood sugar in AFLD compared to controls

The blood sugar in NAFLD patients and healthy control are shown in Table 5. The blood sugar found to significantly increase in NAFLD.

Table 5: Fasting blood sugar in NAFLD compared to controls.

Parameters	NAFLD (n=137)	Controls (n=150)	P value
Blood Sugar (mg)	152.30±57.05	99.93±20.93	p<0.001

Data are presented as mean ± SD, p <0.05 was considered as significant level.

3.6 Liver function tests in AFLD compared to controls

The liver function test- AST, ALT, ALP, GGT, and total bilirubin, in AFLD patients and healthy control are shown in Table 6. The AST, ALT, ALP, GGT, and total bilirubin were found significantly increased in AFLD.

Table 6: Liver function test in AFLD as compared to controls

Parameters	AFLD (n=149)	Controls (n=150)	P value
ALT (IU/L)	116.64±34.7	26.81±7.54	p<0.001
AST (IU/L)	112.98±31.64	28.56±8.82	p<0.001
GGT (IU/L)	120.85±65.47	25.83±9.07	p<0.001
ALP (IU/L)	185.23±46.18	81.56±22.57	p<0.001
T.B.(mg/dl)	0.74±0.24	0.61±0.15	p<0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level.

3.7 Liver function tests in NAFLD compared to controls

The liver function test- AST, ALT, ALP, GGT, and total bilirubin, in NAFLD patients and healthy control are shown in Table 7. The AST, ALT, ALP, GGT, and total

bilirubin were found significantly increased in NAFLD as compared to healthy controls.

Table 7: Liver function tests in NAFLD as compared to controls

Parameters	NAFLD (n=137)	Controls (n=150)	P value
ALT (IU/L)	75.59±24.81	26.81±7.54	p<0.001
AST (IU/L)	75.42±24.62	28.56±8.82	p<0.001
GGT (IU/L)	118.34±38.43	25.83±9.07	p<0.001
ALP (IU/L)	107.99±41.72	81.56±22.57	p<0.001
T.B.(mg/dl)	0.73±0.19	0.61±0.15	p<0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level.

3.8 Plasma LTB4 levels in ALFD compared to controls

Plasma LTB4 levels in AFLD and healthy control are shown in Table 8. The plasma LTB4 levels significantly increased in AFLD as compared to healthy controls.

Table 8: Plasma LTB4 levels in ALFD compared to controls

Parameters	AFLD (n=149)	Controls (n=150)	P value
LTB4 (ng/dl	779.35±218.7	152.60±43.45	p<0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level.

3.9 Plasma LTB4 levels in NALFD compared to controls

Plasma LTB4 levels in NAFLD and healthy control are shown in Table 9. Plasma LTB4 levels are markedly significantly increased in NAFLD as compared to healthy controls.

Table 9: Plasma LTB4 levels in NALFD compared to controls

Parameters	NAFLD (n=137)	Controls (n=150)	P value
LTB4 (ng/dl)	723.7±110.18	152.60±43.45	p<0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level.

3.10 Plasma LTB4 levels in ALFD compared to NAFLD

Plasma LTB4 levels in AFLD and NAFLD are shown in Table 10. Plasma LTB4 levels significantly increased in AFLD as compared to NAFLD.

Table 10: Plasma LTB4 levels in AFLD compared to NALFD

Parameters	AFLD (n=149)	NAFLD (n=137)	P value
LTB4 (ng/dl)	779.35±218.7	723.7±110.18	p<0.07

Data are presented as mean ± SD, p < 0.05 was considered as significant level.

4. Discussion

In the present study, we have showed that the height, weight and BMI were increased in NAFLD patients then AFLD. Body mass index (BMI) is an independent predictor of fat infiltration of the liver, and it is used to screen for weight that may lead to problem of health [11]. Pang Q *et al*., (2015) have reported that the increased BMI a strong association between obesity and NAFLD risk in the population compared with the western population [12]. The fasting blood sugar was found to be no statistically significant (p<0.001) in AFLD and significantly increased in NAFLD as compared to controls. Similar results were reported by Babu Rao *et al* [13].

In our study, we have shows the serum total bilirubin, AST, ALT, ALP, were found significantly increased (p<0.001) in AFLD and NAFLD as compared to controls in both genders. Severity of liver damage is often converted to the amount of alcohol consumption in patients with a history of heavy alcohol abuse [14]. However, liver disease doesn't only depend to the amount of alcohol consumption but also depend on the type II diabetes. Obesity is also play an important role in the development of liver disease. Haring R *et al* [15] showed that γ-glutamyltransfrase levels are frequently increased in NAFLD and correlated with increase risk for mortality and also a predictor of fibrosis in NAFLD patients. Tahan V *et al* [16] have also found that the levels of plasma GGT were significantly (p<0.001) increased in ALD.

In the present study, we have shows that the level of LTB4 was found to be statistically significant **(**p<0.001)) in study groups as compared to the control group. A previous study shown that the susceptibility to alcohol is related to different types of dietary fat and population with a high intake of polyunsaturated fat had a higher than expected rate for alcoholic liver disease [17]. Furthermore, the production of LTB4 by nonparenchymal liver cell was greater. The histological severity of liver injury was correlated with plasma levels of LTB4. Shibayama Y *et al* [18] has suggest that obesity associated LTB4 production by primary tissue cell such as adipose tissues, hepatocytes, myocytes, and endothelial cells, or other tissue cell could be an early trigger for the chronic inflammatory state of obesity. LTB4 directly caused of IR in muscles and also hepatocyte cells. This component of IR is independent effects of LTB4 to tissue inflammation. Therefore LTB4 production is part of an inflammatory response, and can be locked at as a direct mechanism of insulin resistance (IR) operates in both myocytes and hepatocytes [19, 20]. We have shows that the plasma LTB4 level were significantly increased in ALD compared to NAFLD group. The very scanty research work about LTB4 in alcoholic and nonalcoholic liver diseases.

5. Conclusion

In the present study the body mass index (BMI), glucose, and other biochemical parameter are associated with the development and prognosis of liver disease. Leukoterine B4 (LTB4) is most important biomarkers of liver disease, therefore these markers are may be useful for detection of liver diseases.

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