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International Journal of Current Research
Vol. 6, Issue, 08, pp.8159-8162, August, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

CIRCULATING VISFATIN AND PROINFLAMMATORY CYTOKINE LEVELS IN NONALCOHOLIC FATTY LIVER DISEASE

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ARTICLE INFO

Article History:
Received 17th May, 2014
Received in revised form
06th June, 2014
Accepted 27th July, 2014
Published online 31st August, 2014

Key words:

Fatty liver,
Nonalcoholic steatohepatitis,
Tumor necrosis factor–α,
interleukin-6,
interleukin-8.

ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease, ranges from simple steatosis to nonalcoholic steatohepatitis, fibrosis and cirrhosis. Several adipocytokines such as leptin and adiponectin have been implicated in the pathogenesis of NAFLD. However, there are only few data concerning visfatin.

Objective: This study is aimed to evaluate the relationship among circulating visfatin and proinflammatory cytokines in NAFLD.

Material and Methods: The study includes 64 NAFLD and 22 healthy controls with no evidence of liver disease were included. NAFLD was clinically diagnosed on the basis of USG and ALT levels. Serum Visfatin levels were measured using ELISA kit, tumor necrosis factor-α, interleukin-6 and interlukin-8 were measured by Flow Cytometer (BD FACS Array II). Fasting blood glucose, lipid profile and liver enzyme were measured using auto analyzer (Randox). The data were analyzed by SPSS 13.0 version and P value<0.05 were consider significant. Results: Serum visfatin concentration in the NAFLD group (6.38±5.18ng/ml) was significantly lower than in controls (24.62±15.14ng/ml) (P<0.001). There was no correlation between visfatin and anthropometric parameters, TNF-α, IL-6, and IL-8.

Conclusion: The results of this study indicate that increased levels of proinflammatory cytokines TNF-α, IL-6, IL-8 and decreased levels of Visfatin are independently associated with NAFLD.

INTRODUCTION

Non-alcoholic fatty liver disease is the most common cause of liver disease worldwide, ranges from simple steatosis to nonalcoholic steatohepatitis and leading to fibrosis and potentially cirrhosis (Bedogni *et al*., 2005; Vernon *et al*., 2011). The prevalence of NAFLD is 20% in western and non-western countries (Bjornsson *et al*., 2007) and in India the prevalence is (Duseja 2010). The prevalence is increase to 58% in obese individuals and can be as high as 98% in non diabetic obese individuals (Machado *et al*., 2006).Visceral adipose tissue increases the risk for NAFLD in both obese and non obese individuals (Van der poorten *et al*., 2008). Many literatures have demonstrated the role of adipose tissue derived adipo cytokines in the pathogenesis and in the progression of NAFLD (Mulhall *et al*., 2002; Mirza *et al*., 2011; Genc *et al*., 2013; Preiss *et al*., 2008).

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Visfatin, a novel adipo cytokine mostly expressed in visceral adipose tissue. It is also known as nicotinamide phosphoribosyl transferase (NAMPT) and pre-B-cell colony-enhancing factor 1 (PBEF-1). However, it is also found in skeletal muscle, liver, bone marrow and lymphocytes, where it was initially identified as pre-B-cell colony-enhancing factor (PBEF). It regulates the production of pro-inflammatory cytokines in human monocytes and also induced the expression of anti-inflammatory cytokines (Tilg *et al*., 2008; Moschen *et al*., 2010). The biological role of visfatin is poorly understood. Conflicting results have been reported with respect the potential link between NAFLD and Visfatin (Akbal *et al*., 2012; Auguet *et al*., 2013). The purpose of the present study was to investigate the circulating serum visfatin and pro-inflammatory cytokine levels in NAFLD.

MATERIALS AND METHODS

This study was conducted in adults attending the Asian Institute of Gastroenterology, Hyderabad, in a year 2010-2012. The study includes 64 NAFLD and 22 healthy controls with no evidence of liver disease were included. NAFLD was clinically diagnosed on the basis of USG and ALT levels.

For control and NAFLD subjects, the exclusion criteria were hepatitis B, C, cytomegalovirus, Epstein-Barr infections, monogram-specific auto antibodies, alcohol consumption, diabetes mellitus, intolerance fasting glucose, medication (and diabetic drugs, blood-pressure-lowering medication, and statins), and hereditary defects (iron and copper storage diseases and alpha 1-antitrypsin deficiency). The study was approved by an institutional ethics committee, Asian institute of gastroenterology.

All participant's height, weight, waist, Hip values were measured and their body mass index (BMI) was calculated. Glucose, hemoglobin %, total cholesterol, high density lipoprotein (HDL), triglyceride and ALT, AST were studied with Randox auto analyzer (Randox, UK). Insulin levels were measured with Human Elisa- by Mercodia - USA. Serumvisfatin levels were measured by human visfatin ELISA kit, Adipogen, India.Tumor necrosis factor–α, interleukin-6 and interleukin-8 were measured by BD FACS Array II, BD Biosciences, USA.Insulin resistance was calculated by HOMA-IR formula (=fasting insulin value× fasting blood glucose/22.5) (Wallace *et al*., 2004).

Statistical Analysis

The SPSS software package version 19 for Windows was used for statistical analysis. Data are presented as the mean ±standard deviation.P values< 0.05 was considered significant.

RESULTS

Clinical characteristic data of the NAFLD subjects and controls subjects included in the study are summarized in Table 1. The NAFLD subjects have higher (height, weight, BMI, waist, Hip, glucose, Hb%, total cholesterol, TG, ALT and AST) than those of control subjects (p< 0.05 for all).

In contrast, the control subjects have higher HDL cholesterol levels than NAFLD patients. In addition, TNF, IL-6 and IL-8 levels were higher in NAFLD subjects when compared control subjects (13.83±5.42 vs 2.67±0.75, 7.55±19.32 Vs 1.66±0.37, 41.82±80.46 Vs 7.87±4.33) P<0.05. However, no difference was found for insulin and HOMA-IR between the two subjects (Table 1). However, statistically significant decrease levels of Visfatin were observed in NAFLD subjects (6.38±5.18 ng/ml) compared to control subjects (24.6±15.14 ng/ml) (p< 0.05).

There was no correlation between Visfatin and BMI, waist circumference, fating glucose, insulin and HOMA-IR (data not shown). Further there was no correlation between Visfatin and IL-6, IL-8 and TNF-α (P=0.412, r==0.104, P==0.223, r=0.154, and P=0.868, r=-0.021)

DISCUSSION

The present study was conducted to evaluate the circulating Visfatin levels in NAFLD. This study demonstrates a significant decline in Visfatin levels in NAFLD. This results corroborates with earlier reports in which circulating Visfatin levels was shown to be decreased in NASH and NAFLD (Gaddipati *et al*., 2010; Dahl *et al*., 2010; Jarrar *et al*., 2008). However, studies report that impaired liver function to decreased circulating Visfatin levels. Study by (De Boer *et al*., 2009) reported decrease plasma of Visfatin in cirrhotic patients with decreasing levels according to disease severity(De Boer *et al*., 2009). Further study by (Kukla *et al*., 2010), showed a negative correlation between serum levels of Visfatin and the degree of hepatic inflammatory activity in chronic hepatitis C virus infection, further suggesting a link between impaired liver function and low circulating Visfatin levels (Kukla *et al*., 2010).

Table 1. Base line characteristics of NAFLD group and control group

	NAFLD (n=64)	Controls (n=22)	P value
Age, y	39.03±8.06	28.41±6.63	<0.001
Weight (kg)	71.54±10.61	58.54±9.60	<0.001
Height (cm)	165.31±5.98	160.59±7.76	<0.004
BMI (kg/m^2)	26.42±3.73	23.00±3.02	<0.001
Waist (cm)	92.50±12.49	84.86±5.45	<0.006
Hip (cm)	95.32±8.85	87.09±5.40	<0.001
Hb%	14.65±1.77	13.31±1.57	<0.002
FBG (mg/dL)	97.34±14.32	78.86±7.70	<0.001
Fasting insulin (mU/L)1	5.08±11.88	14.24±6.39	0.75
HOMA-IR	3.60±2.76	2.77±1.27	0.17
TC (mg/dL)	186.25±37.61	180.45±36.93	0.53
HDL (mg/dL)	38.89±5.07	45.00±1.87	<0.001
TG (mg/dL)	153.67±60.22	138.81±40.91	0.28
Visfatin (ng/ml)	6.38±5.18	24.62±1.55	<0.001
IL-6(pg/ml)	7.55±19.32	1.66±0.37	<0.001
IL-8(pg/ml)	41.82±80.46	7.87±4.33	<0.001
TNF-α (pg/ml)	13.83±5.42	2.67±0.75	<0.001
ALT (IU/L)	67.35±4.96	19.63±2.38	<0.001
AST (IU/L)	45.4375±2.66	18.68±1.93	<0.001
ALT/AST	0.77±0.30	0.95±0.09	<0.001

Table 2. Correlation between Visfatin and cytokines

Variable	r-value	p-value
Visfatin and IL-6	0.104	0.412
Visfatin and IL-8	0.154	0.223
Visfatin and TNF-α	-0.021	0.868

Further study in animal modeldemonstrated the hepatic regulation of visfatin in wild-type mice and peroxisome proliferators-activated receptor (PPAR) alpha $^{-/-}$ mice as well as in hepatocytes (Dahl *et al*., 2010); thisstudy showed that PPAR alpha activation and glucose may be involved in the down-regulation of hepatic NAMPT/visfatin expression in NAFLD (Dahl *et al*., 2010). NAMPT/visfatin was located to hepatocytes, and *in vitro* studies showed that NAMPT/visfatin exerts antiapoptotic effects in HERK 293 cells and Huh 7 hepatocytes, involving enzymatic synthesis of nicotinamide adenine dinucleotide (Dahl *et al*., 2010). In the present study we did not find any association between Visfatin levels and BMI or waist circumference, fasting glucose, insulin, HOMA-IR, fasting lipid parameters (data not shown). Study by (Alghasham *et al*., 2008)also did not find association between Visfatin and BMI, insulin and HOMA-IR. Similarly, studies by(Gaddipati *et al*., 2010) also did not find association between Visfatin and fasting glucose, cholesterol and triglycerides.

Our study reports increased levels of TNF-αin NAFLD; implicating its role in the NAFLD pathogenesis. Earlier studies demonstrate a potential role of TNF alpha in NAFLD (Gaddipati *et al*., 2010; Hui *et al*., 2004; Kugelmas *et al*., 2003). Interestingly, visfatin expression is regulated by cytokines that promote insulin resistance, such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and lipopolysaccharide (Ognjanovic *et al*., 2001; Fukuhara *et al*., 2005) clearly suggested an endocrine role for Visfatin. Our study reports increased IL-6, levels observed in NAFLD patients compared to healthy controls. Contrary to these findings, increased IL-6 levels in moderate steatosis to NASH were observed which is in agreement with previous study which shown increases in both IL-6 and IL-6R levels in NASH patients (Gaddipati *et al*., 2010; Migita *et al*. 2006). However the role of IL-6 in the pathogenesis of NAFLD is not clear.Our study show increased levels of IL-8 in NAFLD compared to non NAFLD group. This results of this study was corroborates with earlier reports in which IL-8 levels were shown to be increased in (Bahceciouglu *et al*., 2005;Torer *et al*., 2007; Jarrar *et al*., 2008). Further, IL-8 levels were independently associated with NASH (Jarrar *et al*., 2008). These observations suggest a role of visfatin and proinflammatory cytokines in promoting NAFLD.

The present study has certain limitations. Firstly, major limitation of our study was the absence of liver biopsy and the diagnosis of NAFLD was based on liver ultrasonography. It has been argued that other methods; such as magnetic resonance spectroscopy and liver biopsy specimen are better tool for defining NAFLD,and could be used as "gold standard". Secondly, the present study was carried out in limited sample size.

Conclusion

In conclusion, the results of this study indicate that increased levels of proinflammatory cytokines TNF-α , IL-6, IL-8 and decreased levels of Visfatin are independently associated with NAFLD. However, more studies in large population are needed to integrate these results.

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