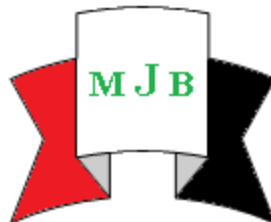


Detection of AMP Deaminase Activity in Patients with Fatty Liver in Hilla City

Sawsan Hassan Kadhum
Babylon Technical Institute



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Abstract

In this study, 35 patients with fatty liver were included, Also, 20 healthy individuals were subjected to this study as control group.

It was found that all patients with fatty liver significantly have increased levels of triglyceride and cholesterol where as there was no significant in increasing of uric acid levels.

Besides, AMP deaminase was also studied .It was observed that enzyme activity was increased significantly in all patients with fatty liver when compared with control group.

Metformin was also used at different concentrations to show its *in vitro* effect on enzyme activity. It was seen that metformin had reduced AMP deaminase activities at 5 mM and 10 mM of Metformin where as the activity was entirely overcome above 20 mM .

Key Words: AMP Deaminase, Fatty Liver, Hilla

التحري عن فعالية انزيم ادينوسين احادي الفوسفات دي امينيز في المرضى المصابين بمرض الكبد الدهني في مدينة الحلة

الخلاصة

تضمنت هذه الدراسة ٣٥ مريضاً يعانون من مرض الكبد الدهني و ٢٠ شخصاً من الأصحاء كمجموعة سيطرة . لقد وجد إن جميع المرضى الذين يعانون من مرض الكبد الدهني غير الكحولي , لديهم زيادة معنوية في مستويات الكولسترول الكلي وثلاثي الكليسرات وزيادة غير معنوية في مستوى حامض اليوريك . إضافة لذلك فقد درست فعالية انزيم الادينوسين احادي فوسفات دي امينيز في مصول مرضى الكبد الدهني من الأصحاء وقد وجد ان هناك زيادة معنوية في مستويات الإنزيم في مصول المرضى إذا ما قورنت بمصول الأصحاء. كما تمت دراسة تأثير المتفورمين وبتراكيز مختلفة على فعالية الإنزيم وقد وجد إن العقار يؤدي إلى انخفاض واضح في فعالية الإنزيم عند التراكيز ٥ ملي مول , ١٠ ملي مول , إلا إن الفعالية تخفت في التراكيز فوق ٢٠ ملي مول.

Introduction

Fatty liver or Non-alcoholic hepatic steatosis is a condition characterized by the accumulation of excess fat in the liver particularly triglyceride (TG), not caused by alcohol abused but associated with obesity and the metabolic syndrome [1].

There has been a progressive increase in obesity, type 2 diabetes, dyslipidemia and fatty liver throughout the world [2]. In particular, there has been an increasing frequency of non-alcoholic hepatic steatosis in subjects with obesity or insulin resistance that may progress to chronic liver diseases

[3]. Fatty liver disease affects all race and ethnic groups and has no age or sex prediction [4]. It compasses a wide spectrum of diseases ranging from simple steatosis characterized by hepatic lipid accumulation in the form of TG to non-alcoholic steato hepatitis characterized by association of lipid accumulation with evidence of hepatocyte injury, inflammation and various degree of fibrosis [5]. Fatty liver is associated with nucleotide turnover, loss of ATP and generation of adenosine mono phosphate, (AMP) [4].

However, there are two enzymes that are so essential in fat accumulation, AMP deaminase and Amp dependent kinase, and the increasing of AMP deaminase and inhibition of Amp kinase will give rise to fatty liver, where as inhibition of AMP deaminase and activation of AMP kinase will prevent the accumulation of fat and then prevents the occurrence of fatty liver [3,6]. One the most common inhibitor for AMP deaminase is metformin [7].

The aim of this study is to detect AMP deaminase in the sera of patients with fatty liver and the in vitro effect of metformin on this enzyme activity.

Materials and Methods

1-Patients

Thirty five patients with non-alcoholic fatty liver cases without hepatitis who attending Al-hayat Private Lab.(Hilla-Iraq) were included in this study.

The diagnosis of non-alcoholic fatty liver cases was based on ultrasonography which was not confirmed by liver biopsy.

The age of patients ranging from 42 – 66 years old from both sexes. Body mass index (BMI) was also estimated at a value 27.3 ± 2.88 .

Serum samples were obtained from all patients. Twenty healthy individuals were also included as control group.

2-Biochemical tests.

Uric acid, triglyceride and cholesterol were estimated in the sera of patients according to manufactured company (Cromatest , Spain).

3-Determination of AMP deaminase activity :

AMP deaminase (AMP amino hydrolase EC 3.5.4.6) activity was carried out using the procedure mentioned by [8]. Adenosine monophosphate (5-AMP) was used as substrate and phosphate buffer (0.05M) at pH 6.5 containing KCl (0.025M) was also used for determination enzyme activity.

The assay system was accomplished in duplicate and the enzyme activity was estimated in (unit/L) according to equation mentioned by the same reference [8]. Metformin (Merk – santé, France) was used at different concentration (5,10,15,20,25 mM) to show its in vitro effect on enzyme activity. This was added with substrate solution pre-addition the serum.

Results and Discussion

In this study, 35 patients with fatty liver were subjected in this study.

Uric acid, triglyceride and cholesterol were estimated in the serum sample of those patients and compared with those samples obtained from control group.

It was observed that triglyceride and cholesterol levels were significantly [$P>0.001$] increased in all patients sample where as uric acid levels were also increased but not significantly (Table 1). These results were identical to those obtained by [5] who found that the

patients with fatty liver had significantly higher values of total cholesterol and serum triglycerides.

Table 1 Some biochemical tests for patient with fatty liver

Tests	Mean ± SD		P value
	Patient (35)	Control (25)	
Uric acid mg/dL	6.4 ± 1.9	4.1 ± 1.3	0.01
Triglyceride mg/dL	237 ± 39.8	66 ± 22.2	0.001
Cholesterol mg/dL	233 ± 18.6	145 ± 10.9	0.001

It was indicated that the most important lipid that accumulate in the hepatocytes is triglyceride which is responsible for fatty liver disease [5,6]. AMP deaminase was also estimated, it was found that there was high levels of

this enzyme activity when compared to control group. This increasing of ADP deaminase was highly significant (P>0.001). (Table 2)

Table 2 AMP deaminase activities in patients with fatty liver

Tests	Mean ± SD		P value
	Patient group (35)	Control group (25)	
AMP deaminase U/L	3.66 ± 0.92	1.09 ± 0.74	0.001

An increasing in AMP deaminase catalyzes the degradation of AMP into IMP and then to uric acid. The increase of intracellular uric acid is followed by acute rise in the uric acid in the circulation likely due to its release from the liver [9].

However, According to the data obtained in this study, uric acid was increased in some patients with fatty liver, but it's being within normal levels did not mean that it couldn't induces hepatic steatosis through increasing AMP deaminase activity [10].

Moreover, It was stated that uric acid induces hepatic steatosis by generation of mitochondrial oxidation stress, which results in the inhibition of Aconitase enzyme in the Krebs cycle,

resulting in the accumulation of citrate and stimulation of ATP citrate lyase and fatty acid synthesis leading to de novo lipogenesis [11].

Besides, the over expression of AMP deaminase blocks fatty acid oxidation and increases fat accumulation where as silencing of this enzyme blocks fat accumulation [9,10].

So, in distinct experiment, since metformin is one of the most important drug for preventing fatty liver through its ability to inhibit AMP deaminase, various concentrations of this drug were done to show its *in vitro* effect on enzyme activity.

It was noticed that metformin diminishes the activity of this enzyme at a concentrations below 20 mM and the

activity was entirely inhibited at the concentration above 20 mM (Table 3).

Table 3 *In vitro* effect of metformin on AMP deaminase activity :

Tests	Metformin concentration					
	0 mM	5 mM	10 mM	15 mM	20 mM	25 mM
AMP deaminase U/L	3.41	1.95	0.52	0.10	0.00	0.00

This will indicate that metformin should be given for patients suffering from fatty liver particularly those with high body mass index (BMI). Metformin (Commercially is named glucophage) is currently the most widely used drug for type 2 diabetes, and recently it was discovered that metformin causes an activation of an enzyme AMP –activated kinase (AMP kinase) which prevents fatty liver disease through its preventing of fat accumulation [12].

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