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Iron and its related parameters in serum and saliva of Iraqi patients with non-alcoholic and alcoholic liver disease

Abstract

Background: The liver is the major site for production of proteins that maintain systemic iron balance; it is a storage site for excess iron. The aim of this project is to look for the differences in iron status in patients with non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD) compared to that of control and to assess whether saliva can be used as an alternative to serum to measure this status.

Methods: The present study included four groups of Iraqis male, (18 patients with NAFLD), (18 patients with ALD), (30 non-smokers and non-alcoholic control), and (20 smokers non-alcoholic control). The used samples of the study were saliva and serum and the measured parameters included [iron], total iron binding capacity (TIBC), and unsaturated iron binding capacity (UIBC), [transferrin], transferrin saturation, and [ferritin].

Results: The results in comparison with that of the control group's showed:-

a. For NAFLD, there was a highly significant differences in salivary (UIBC, transferrin saturation), serum [ferritin], with a significant differences in salivary TIBC, [transferrin]. And no significant differences in serum ([iron], TIBC, UIBC, [transferrin], and transferrin saturation), and saliva ([iron], and [ferritin]).

b .For ALD, there was a highly significant differences in serum [ferritin], with a significant differences in TIBC, UIBC, and [transferrin] in serum with a non-significant differences in salivary ([iron], TIBC, UIBC, [transferrin], and transferrin saturation), as well as serum ([iron], transferrin saturation, and [ferritin].

c. The results of the correlation between the changes of the studied parameters in serum and saliva indicated that only for control 1 group (which consisted of non-smokers and non-alcoholic healthy individuals) a significant correlation was found in [iron].

Conclusion: An iron overload was found in serum of ALD patients with absence of this overload in NAFLD patients. Moreover, in saliva, the results of ALD patients indicated absence of iron overload. While in saliva of NAFLD patients, an iron overload was observed. Saliva cannot be used as an alternative diagnostic fluid sample in both ALD & NAFLD patients to measure the variations in the iron parameters included in the present study.

Keywords: non alcoholic fatty liver disease, alcoholic liver disease, iron overload, serum, saliva

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Introduction

The most serious diseases of the liver are viral hepatitis and fatty liver diseases.[1] The latter one is generally classified into two categories, each with a different etiology, alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD). Both lead to pathological triglyceride accumulation within liver cells, that cause liver cirrhosis and in some cases developed to hepatocellular carcinoma where it cannot repair itself.[2,3]

The liver is the major site for proteins production that maintains systemic iron balance; it is a storage site for excess iron. As well as it has an important role in the mobilization of iron from hepatocytes to the circulation to meet the body metabolic requirements.[4] In the human body, 75-80% of iron is found in hem group of hemoglobin, and a moderate amount in myoglobin.[5,6] Iron level is regulated by a hepatic peptide hormone, hepcidin.[5]An elevated iron level stimulates hepcidin synthesis, which decreases the iron exporter ferroportin in macrophages and intestinal cells and thus reduces serum iron.[7]

The increase in the expression of intestinal iron transporters leads to increased intestinal iron absorption, and hence to increase body iron index.[8–10] Several studies measured serum iron parameters in ALD patients.[11,12] It has been reported that alcohol consumption increases the transfer of iron from the intestine into the circulation.[13] The association between body iron stores and risk of diabetes have been prospectively examined by several studies.[14,15] And elevated iron stores below the levels of known iron overload syndromes have also been implicated in the etiology of diabetes.[16,17] On the other hand, the role of hepatic iron in NAFLD pathogenesis has largely focused on the generation of oxidative stress by iron.[18] It is worth to mention

that so far no study is found in the literature which deals with iron related parameters in saliva of both NAFLD, and ALD patients. The present study focused on serum and salivary iron status in NAFLD, and ALD patients. The measured parameters included [iron], total iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC), [transferrin], transferrin saturation, and [ferritin].

Materials and methods

The participants of the current study involved 86 individuals: 18 Patients with non-alcoholic fatty liver disease (NAFLD), who were diabetic, and non-smokers, and 18 patients with alcoholic liver disease (ALD) patients who were alcoholic and smokers for at least 20years. For comparison purpose, two groups of age-matched healthy controls were also included in the study. The first control group was consisted of 30 non-smokers and non-alcoholic to be used as a control for NAFLD patients, while the second control group included 20 smokers and non-alcoholic, healthy individuals to be used as a control for ALD patients. The baseline examination included a personal health interview, a health questionnaire linked to their study along with their personal full details history including medical history, laboratory evaluation, a radiographic study including computed tomography (CT), magnetic resonance imaging (MRI), as well as, the results of liver biopsy examination. The ethics Committee of Baghdad University/ College of Science has approved the study project.

Inclusive criteria of the participants

The NAFLD patients were diabetic with a mean value of blood sugar concentration (274.88±66.27mg/dl). While the ALD patients were non-diabetic. The control groups included in the present study were age-matched individuals who fulfilled inclusion criteria.

Exclusion criteria

The excluded criteria for both ALD, and NAFLD groups were: non-alcoholic cirrhosis due to Wilson disease, hepatitis B, C, α1-antitrypsin deficiency, haemochromatosis, obese person, and vitamin supplements usage. An, alcoholic smokers NAFLD patients as well as, non smokers and diabetic ALD patients were excluded from the present study.

Samples

After overnight fasting, 10mL of blood were withdrawn in serum tube then centrifuged at (3000xg) for 5 minutes. At the same time, from the same individual, unstimulated saliva samples were taken and the participants were asked to rinse their mouth with saline before collecting the saliva samples. The saliva samples were centrifuged for 10minutes at (2400×g). The sera and saliva samples were collected and kept frozen to be used for the determinations of the different studied parameters.

Chemicals

All chemicals used in this study were of the highly analytical grade.

Methods

Determination of total iron binding capacity (TIBC)

The sample (serum and saliva) were treated with excess Fe (II) to saturate the iron binding sites on transferrin. The excess unbound Fe (II) was precipitated by the addition of magnesium carbonate and the iron content in the supernatant is measured to give the TIBC.[19] The results were expressed as (µg/dl).

Determination of iron concentration

Iron in serum and saliva was determined using Randox kit. Ferrozine reacts with ferrous ions in the sample to form a colored complex. Ferrous ions may be found as free iron, and the ferric ions (which are bound to transferrin that released in an acidic medium) reduced to ferrous ions by ascorbic acid. The results were expressed as (µg/dl).

Determination of transferrin

Transferrin was estimated indirectly from the following equation.[19]

Transferrin (µg/dl)=0.7xTIBC (µg/dl)

And from the following equation, the percentage of saturation of transferrin with iron was calculated:

Saturation of transferrin %=(Iron concentration/TIBC)x100

Determination of ferritin concentration

Ferritin levels were estimated by a solid phase enzyme-linked immunosorbent assay technique (ELISA) using a commercially available Randox kit. The concentration of ferritin in the saliva and serum was expressed in ng/mL.

Statistical analysis

The data were analyzed using SPSS by Licensed Materials version 22 computer software. Data in this study was presented as Mean±Standard deviation (Mean± SD) using Independent-samples T-Test to compare the mean. A ($p<0.05$) value, was approved as statistically significant, while a ($p<0.001$) value, was approved as a highly significant.

Results and discussion

According to the classification of patients and control groups, the results of the measured parameters in patients groups were compared with that of their corresponding controls groups. The results in Figure 1 show that there is no significant differences in iron concentration in serum and saliva of patients groups compared to that of their corresponding control groups ($p>0.05$).

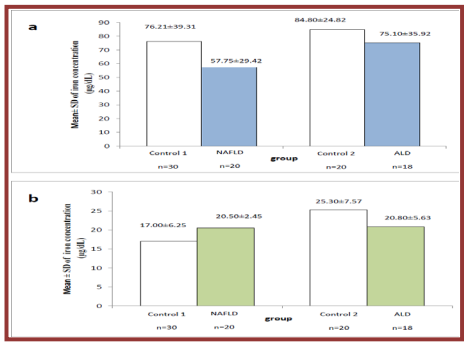

Figure 1 Mean value±SD of [iron] concentration in (A) Serum. (B) Saliva of all studied groups.

Total iron binding capacity (TIBC) which represents the maximum concentration of iron that can be bound by protein (transferrin) was measured. Figure 2 shows the comparison of (TIBC) in serum and

saliva of all groups respectively. It is clear that there is no significant difference in serum (TIBC) of NAFLD group in comparison to that of its control group ($p>0.05$), while a significant decrease was observed in that of serum of ALD group compared to that of its control group ($p<0.05$). A significant decrease in the salivary TIBC of NAFLD group is noticed compared to that of the control group ($p<0.05$), with no significant differences in that of saliva of ALD patients. The TIBC concentration depends on transferrin which is produced in the liver. It has been reported that, due to the decreased transferrin synthesis in case of chronic liver disease, (TIBC) progressively decreased with the disease severity.[20]

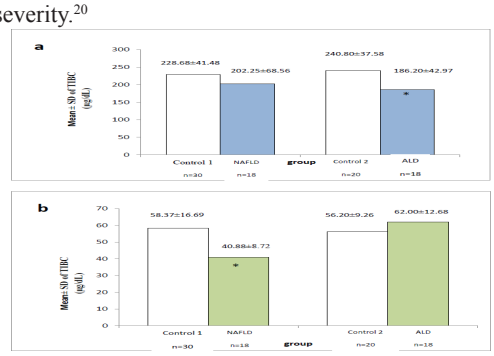

Figure 2 Mean value±SD of TIBC in (A) Serum. (B) Saliva of all studied groups.

*The difference is significant at the 0.05 level.

The other parameter related to iron status is unsaturated iron binding capacity (UIBC), which was calculated by subtracting iron concentration from (TIBC). Figure 3 shows the results of UIBC in all studied groups. No significant difference is found in (UIBC) of the patients with NAFLD compared to that of its control group ($p>0.05$), while a significant decrease is clear in case of ALD group compared to its control group ($p<0.05$). In saliva a highly significant decrease was observed in that of NAFLD group ($p<0.001$), with no significant difference in that of ALD group compared to its control group ($p>0.05$).

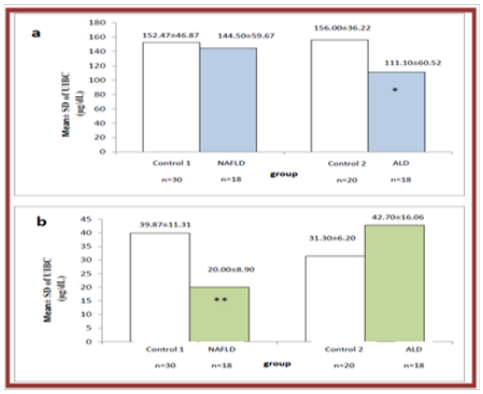

Figure 3 Mean value±SD of UIBC in (A) Serum. (B) Saliva of all studied groups.

*The difference is significant at the 0.05 level.

Transferrin, an iron carrier protein which binds iron in soluble form (Fe^{3+}), is synthesized in the liver, and is highly correlated with TIBC. The results of transferrin level measurement in serum and saliva in all studied groups are presented in Figure 4. These results indicate that there is no significant difference between serum [transferrin] of NAFLD group compared to its control group ($p>0.05$), with a significant decrease in that in the serum of ALD group in comparison to that of its control group ($p<0.05$). Meanwhile, in saliva of these patients, a significant increase ($p<0.05$) is obvious in [transferrin] of NAFLD, with nonsignificant differences in saliva of ALD group in comparison with their corresponding control groups.

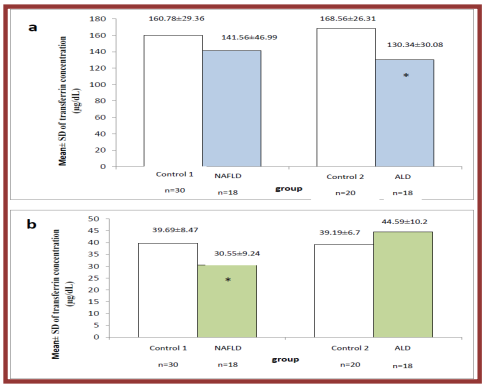

Figure 4 Mean value±SD of [transferrin] in serum and saliva of all studied groups.

*The difference is significant at the 0.05 level.

Transferrin saturation clarifies the presence of transferrin which saturated by iron. Figure 5 shows that there is no significant differences in transferrin saturation in serum and saliva of all groups ($p>0.05$) except for that in the saliva of NAFLD group where a highly significant increase is clear in this patient group in comparison with its corresponding control group ($p<0.001$). Ferritin, an iron storage protein, store iron as insoluble form (Fe^{2+}). It acts as an antioxidant because it inhibits the toxic effect of free iron.[21] The measured ferritin concentration in serum and saliva in all groups is illustrated in Figure 6. In serum, a highly significant increase in this parameter is obvious in both patients groups compared to that of their corresponding control groups ($p<0.001$). Meantime, no significant differences were observed in that of saliva of both patients groups ($p>0.05$).

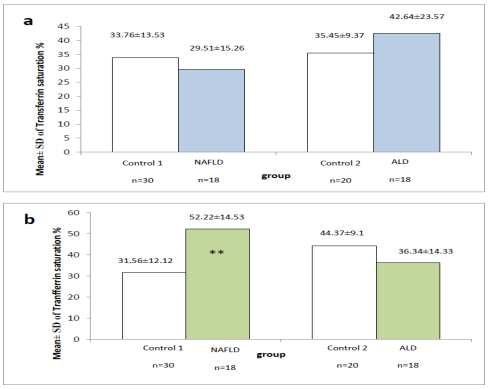

Figure 5 Mean value±SD of transferrin saturation in (A) serum and (B) saliva of all studied groups.

**The difference is a highly significant at the 0.001 level.

Alcohol abuse and smoking induces an increase in body iron levels, which has been suggested be due to the elevation of the iron absorption from the food in the gastrointestinal tract.[20,22,23] The results of measured iron parameters showed a decrease in serum TIBC, and

an increase in serum [iron] in ALD compared to those in NAFLD. These results agree with many previous studies such as a study by Jurczyk, et al.[11] in Poland and Khare et al.[20] in India. The causes of the progression of liver fibrosis in chronic liver disease might be due to the regular consumption of alcohol which has been reported to be responsible for the disruption of normal iron metabolism in humans, this cause an elevation deposition of iron in the liver.[24] Such deposition state seems to be due to the effect of alcohol on hepcidin, which its hepatic expression is down regulated by alcohol, this leads to elevated expression of the iron transporter proteins, divalent metal transport1 (DMT1), and ferroportin in the duodenum. The increase in intestinal iron transporter expression leads to increase intestinal iron absorption, and hence to increase body iron indices.[8,9] The other factor is cigarette smoking, which causes tissue hypoxia, that causes inadequate oxygenation of blood circulation and thus results in erythropoiesis and consequent increased production of erythropoietin. This in turn enhances erythropoiesis and increases red cell mass above the normal level.[25] The ultimate results will be an increase in the number of destroyed red cells in the normal turnover process which subsequently increase iron overload that cause hepatocellular damage.[23]

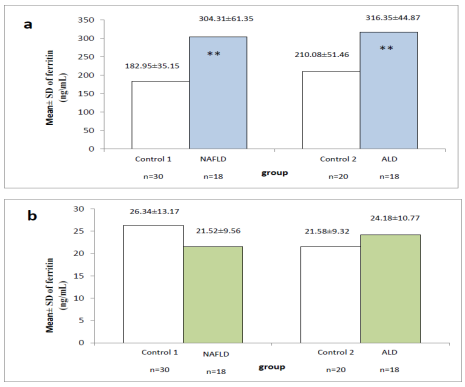

Figure 6 Mean value±SD of ferritin concentration in (A) Serum. (B) Saliva of all studied groups.

**The difference is a highly significant at the 0.001 level.

Serum ferritin concentration is by far the most commonly used indicator of body iron stores in epidemiological studies.[26] The use of serum ferritin in assessing iron stores, however, is complicated because ferritin is also an acute-phase protein that may be elevated in many inflammation condition such as liver disease.[24]

To check if the elevation of serum ferritin levels result from acute, or chronic inflammation rather than iron overload.[15,27,28] The measurement of ferritin concentration and transferrin saturation was carried out. The observed increases in their levels indicate that there is an iron overload in ALD patients. While in NAFLD patients the increase in serum ferritin that accompanied with decrease in transferrin saturation indicate absence of iron overload rather than this, the increase in ferritin here is seems to be due to inflammation.

The results of the present study are consistent with findings from previous prospective studies in several countries reporting high serum ferritin concentrations in NAFLD patients.[15,28–32] On the other hand, liver cells are among other cells which stored ferritin, this means that liver damage for any reason causes leaking of its proteins into the blood & among these proteins is ferritin.[15,33,34] The other reason of increase ferritin concentration may be due to the decrease in the activity of proteins which is required to efflux iron across the liver. This efflux across the basolateral membrane of enterocytes requires the transporter protein ferroportin and a multicopper oxidase (MCOs), which are required to oxidize, exported Fe^{2+} to Fe^{3+} for loading into serum transferrin.[21]

In saliva, the results of ALD patients show the absence of iron overload this conclusion is based on the observed decrease in [iron] and transferrin saturation in Figure 1& Figure 5 respectively. On the other hand, in the saliva of NAFLD, the elevation in [iron] and transferrin saturation indicates presence of an iron overload in these patients. To our knowledge, this is the first report concerning the measurement of iron related parameters in the saliva of patients with liver. In order to check the possibility of using saliva as a sample of analysis instead of serum, a correlation between the iron profile in serum and saliva was done and the results is presented in Table 1. It is clear from these results among the measured parameters there is a significant correlation in [iron] only ($p<0.05$) in control 1 group who were nonsmokers and nonalcoholic, with no correlation in all measured parameters in the other studied groups.

Table 1 Personal correlation between serum & saliva of iron parameters in all studies groups

Serum Saliva	Control 1	NAFLD	Control 2	ALD
[Iron]	0.539*	0.067	0.121	-0.478
TIBC	0.204	-0.169	0.227	0.213
UIBC	0.189	-0.165	0.355	0.177
[Transferrin]	0.147	-0.268	0.249	0.093
Transferrin saturation	0.22	-0.456	0.332	-0.143
[Ferritin]	0.046	0.278	-0.575	0.162

Conclusion

From the results of the present study one can conclude that

1. In serum, there is an iron overload in ALD patients, and an inflammation in NAFLD patients
2. In saliva, the results of ALD patients indicated the absence of iron overload, while in NAFLD patients, an overload in iron was present.
3. Saliva of both ALD & NAFLD patients cannot be used as an alternative fluid samples to serum for measurement of the variations in the iron and its related parameters included in the present study. But it can be used for this purpose to measure the changes in [iron] in nonsmokers, nonalcoholic healthy individuals.

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Conflicts of interest

Author declares that there are no conflicts of interest.

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