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Evaluation Hepcidin Hormone on the Level of Iron in Blood and Its Effect for Hepatitis B with Iraqi Patients

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Abstract:

Liver disease, is the term given to any of the diseases or disorders that lead to the fall or loss of the liver's ability to functionThe liver is second largest organ in human body, and has more than 5,00 separate bodily functions including assistance the blood to clot, cleansing the blood of toxins, to convert food into nutrients, to control hormone levels, fight inflammation and disease, and reproduce again after injury and metabolizecholesterol, glucose and iron and control its levels. it is also produces bile Which contributes to digest fatty foods, liver diseaseincreases affecting about one in ten. Liver disease can be hereditary or caused by a variety of factors that damage the liver, in fact, there are many types disease of liver that can be caused by a virus, damage from drugs or chemicals, obesity, diabetes, or an attack from the immune system, when this is the case. These diseases are classified according to the main cause that led to their occurrence in addition to their effect on Liver, and it can be explained accordingly as follows. (Viral hepatitis,fatty liver disease, liver cancer,) This study focused on one types of viral hepatitis, which is, viral hepatitis B. The main variable was hepcidin hormone, It was measured by the ELISA method according to standard procedures at wavelength: (450nm). ALP enzyme was automated measurement at the same wavelength.This study was aimed to evaluate hepcidin level in patients with hepatitis B Virus and its effect on the level of iron and other Parameters, and to assess the liver function enzyme and other biochemical test in Iraqi patients infected with Hepatitis B addition to Healthy individuals.

Keywords: Hepcidin, hepatitis, iron and Liver disease

Introduction

Chronic hepatitis B (CHB) is a common chronic liver disease (CLD) that often advance to cirrhosis and is linked with increased risk of hepatic- cellular carcinoma (HCC) as well as high rates of morbidity and mortality [1]. Therefore, development of practical, noninvasive biomarkers for the oversight of hepatitis B (HBV)-related diseases and their advancement is of great clinical value [2]. Iron overload, which is common in chronic HBV-related diseases, is related with oxidative stress and sequent tissue damage and chronic inflammation in the liver [3]. This related assert the potential role of hepcidin—a key regulator of iron homeostasis primarily produced by

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liver cells—as an indicator of changes in iron metabolism in HBV-related diseases [4]. A better understanding of the link between viral hepatitis, iron overload and disease development is required, and improved clinical oversight and treatment justify [5]. In this inspect, many indices of iron metabolism such as hepatic iron levels, serum iron, ferritin levels, lipid profile, Level of enzymes (Got, Gpt), Urea, and cert have been routinely used as diagnostic tools for iron overload as a risk factor for liver disorder [6]. in particular, hepcidin hormone, which was discovered in 2000 has emerged as a potential marker for fibrosis and cirrhosis owing to its role as a master regulator of systemic iron homeostasis by limiting serum iron levels through the control of iron efflux from cells [7]. However, most of studies recce the utility of hepcidin levels in patients with HBV-related diseases were based on the measurement of the hepcidin precursor pro-hepcidin [8],and the impact of pro-hepcidin on iron metabolism regulation and itscorrelation with hepcidin levels remain indistinct [9]. Increased serumhepcidin levels have been reported in patients with CHB and HCC [10],whereas decreases in serum pro-hepcidin levels have been reported in patients with CHB [11]. Furthermore, reduced hepcidin levels have been reported in patients with CHB, chronic hepatitis C (CHC) infection, alcoholic liver disease (ALD) nonalcoholic fatty liver disease [12,13] and HBV-related cirrhosis [14]. As for hepcidin which is a 25- amino acid peptide. It regulates the iron transport from dietary sources for extra cellular maintenance of iron concentration. Iron gets transported from recycled senescent red cells in macrophages, in the duodenum and are stored in hepatocytes. Hepcidin is responsible for the interaction of iron with its receptors ferroportin - a transmembrane export protein. Ferroportin is abundant in cell surface of reticulo endothelial macrophages and on base lateral membrane of duodenal enterocytes [15]. Hepcidin synthesis can be increased and decreased by iron loading, anemia and hypoxia respectively [16].Hepcidin is considered as a negative regulator of iron absorption and recycling, because it inhibits the release of iron at both the sites by binding to cell surface ferroportin and causing internalization and subsequent degradation [17]. The iron store in our can be indicated by hepcidin [18]. Hemochromatosis genes which encode molecules that regulate hepcidin synthesis any change or mutation in this gene will modify ferroportin and make it less responsive to Hepcidin [19]. Hepcidin interacts directly with the intestinal epithelium. It controls the amount of iron absorbed from the diet by modulating the uptake mechanism of apical membrane [20].Hepcidin is regulated by iron and erythropoietic activity (figure 1), Iron excess stimulates hepcidin production, and increased concentrations of the hormone in turn block dietary iron absorption thus preventing further iron loading, Conversely, hepcidin is suppressed in iron deficiency, allowing increased absorption of dietary iron and replenishment of iron stores [21].

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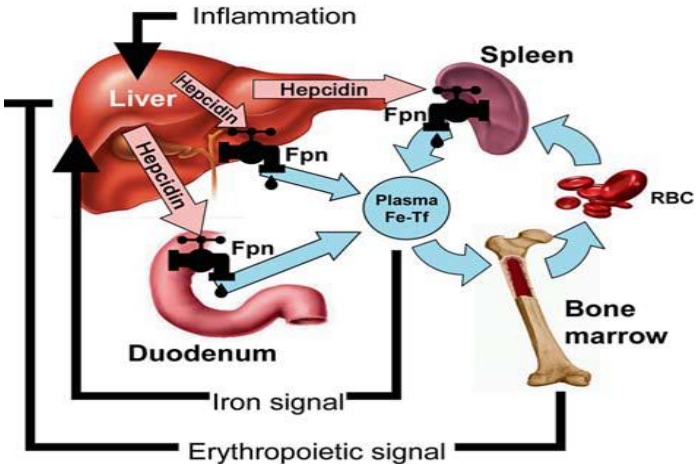

Figure 1: Hepcidin-ferroportin (Fpn) interaction determines the flow of iron into plasma. Hepcidin concentration is in turn regulated by iron, erythropoietic activity and inflammation[21].

Hepcidin is a regulator of iron metabolism. Hepcidin inhibits iron transport by binding to the iron export channel ferroportin which is located on the basolateral surface of gut enterocytes and the plasma membrane of reticuloendothelial cells (macrophages). Hepcidin ultimately breaks down the transporter protein in the lysosomes. Inhibiting ferroportin prevents iron from being exported and the iron is sequestered in the cells [22]. By inhibiting ferroportin, hepcidin prevents enterocytes from allowing iron into the hepatic portal system, thereby reducing dietary iron absorption. The iron release from macrophages is also reduced by ferroportin inhibition. increased hepcidin activity is partially responsible for reduced iron availability seen in anemia of chronic inflammation such as renal failure Any one of several mutations in Hepcidin result in juvenile hemochromatosis. The majority of juvenile hemochromatosis cases are due to mutations in hemojuvelin [23].when reported hepcidin overexpression during iron overload the bioactive form of hepcidin is the 25–amino acid peptide hormone secreted by hepatocytes, the main cell type in the liver Hepcidin inhibits the release of iron into the circulation by postranslationally regulating its cognate receptor ferroportin,hepcidin binding leads to internalization and degradation of the ferroportin the iron transporter expressed on the basolateral membrane of duodenal enterocytes, on macrophages, placental syncytiotrophoblasts, and hepatocytes Cell surface ferroportin deficiency prevents iron from entering the plasma. Reduced iron entry into plasma results in low transferrin saturation and less iron is delivered to the production of erythroblast in addition, reduced hepcidin expression results in increased ferroportin cell surface and increased iron absorption Irregular secretion of Hepcidin leads to an imbalance in iron regulation [24].

Material and methods

Current study incorporated patients 43 with hepatitis B They were compared with 43 healthy controls, Patient samples were collected at Medical City/ Gastroenterology Hospital, and Al-Kadhimiya Teaching Hospital. during the period from Sept 2020

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until Nov 2020.Their ages ranged from 18 to 77 years, healthy individuals age 35-65 years as control group.The Hepatitis patients were diagnosed based on their medical reports, laboratory, and clinical tests for liver disease. Five milliliters of blood samples were taken from each patient. Patients' blood samples were obtained from the needle puncture site for the patients' vein. Serum that obtained was stored at -20℃ until the time of screening. Venous blood samples were also taken from the control group using disposable syringe and centrifuged to obtain serum. Patients with neuropathy, Liver stubbornness, and patients with Hepatitis a, e and d were excluded from the study.

Statistical Analysis

Results of this study were expressed as [mean ± SE], T. test (spss) was utilized to Compare between three studied groups, so T. test less than or equal of 0.05 was considered significant and highly significant, respectively. Also this study expressed as correlation of hepcidin-25 with all parameter, so correlation value between range (±0.5) was considered significant.

Results and Discussion

The mean ± SE of hepcidin is (151.56 ±1.48) pg / mL for patients with hepatitis B. which is less than healthy individuals (187.60 ±4.24) as illustrated in Table 1**.** The patients were into two groups, the first group [G1] is Patients with hepatitis B, and the second group [G2] is the control. **Table 1** showedparameters result of 43th patients with hepatitis B, While **Table 2** showed the results of the age and BMI (body mass index) for the same group.

Serum Hepcidin, Serum Iron and Serum ferritin

In this study investigation the effect of the level of hepcidin in iron overload in patient group, hepcidin hormone where the results show generally there was a significant increased (P ≤0.01) the iron parameter in patients with low level hepcidin.Results of Table (1) and Figure (2) revealed a highly significant decrease in Hepcidin levels in G1 as compared with G2.It shows a significant decrease in sera of iron and ferritin in G2 comparison with G1,Iron and ferritin levels are shown respectively in Figure (3, 4).

Table (1) Results of Hepcidin Iron and ferritin levels in both groups, G1: group of patients with hepatitis B, G2: healthy control group

	Mean ± SE		
Group	Hepc (pg/ml)	Ferritin (mg/dl)	Iron (mg/dl)
G1	151.56 ±1.48	519.67 ±10.94	154.16 ±1.85
G2	187.60 ±4.24	289.60 ±12.27	92.7 ±3.83
P-value G1&G2	**HS**	**HS**	**HS**
	(P≤0.01)		

HS: Highly significant, S: Significant, NS: Not significant.

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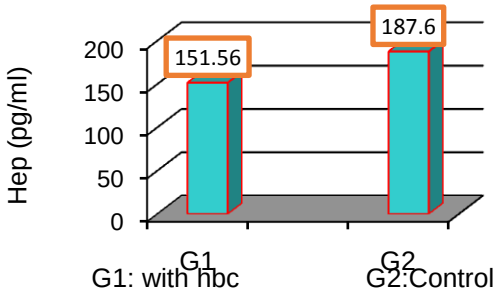
Figure 2: Compare between difference groups in Hep



In the present cross-sectional study, hepatic iron overload was evident in patients with CHB,Advanced fibrosis was significantly associated with the biochemical liver profile,viral load, hepcidin levels and serum iron parameters. In addition, higher ferritin levels were observed in patients with advanced fibrosis than in those with mild fibrosis [25]. Chronic HBV infections are associated with increased production of ROS within the liver, which can function as a component of signal transduction cascades by activating transcription factors including STAT-3, the pathway activating hepcidin transcription in the hepatocytes [26]. IL-6 activity has been shown to be significantly enhanced during acute exacerbation of CHB, and plays an important role in elevating hepcidin production [27].In our study, significantly increased IL-6 levels were found in patients with both CHB and HCC. Accordingly, serum hepcidin levels were also elevated in CHB patients, supporting the notion that elevated IL-6 might stimulate hepcidin expression during HBV infection. However, we did not find any quantitative correlation between IL-6 and hepcidin, possibly due to the complexity of immune response in hepatitis B, where panels of cytokines are expressed, a setting very different from the controlled application of single cytokine or LPS during an in vitro experiment, instead reflecting the net effects of several agonistic and antagonistic cytokines toward a specific target. Thus, the potential effect of a single immune effective molecule found in experiments may be masked in this in vivo setting Hepcidin is gradually being accepted as an important systemic immune response mediator. Inflammation and elevated iron stores are two major stimuli for hepcidin secretion. As the liver is the major source of hepcidin production we examined whether its inflammatory status can affect hepcidin production. In hepatitis, ALT and AST become elevated with the progression of liver disease, likely as a result of direct hepatocellular damage and membrane leakage. In our CHB patients, ALT and AST levels were elevated, as was HBV virus load, indicating our patients were in the active phase of CHB. Hyper ferritinemia is a frequent phenomenon in patients with chronic liver disease, whatever the etiology of the underlying damage [28]

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Figure 2. Compare between difference groups in Ferritin

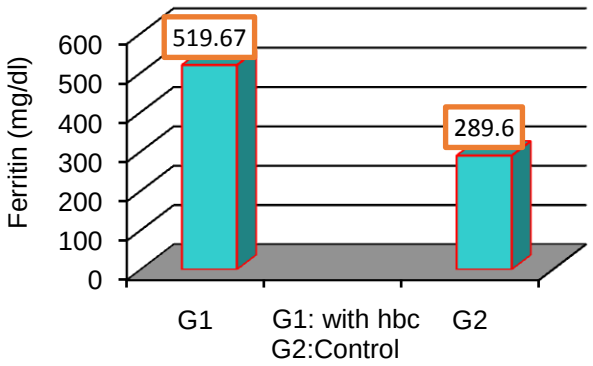
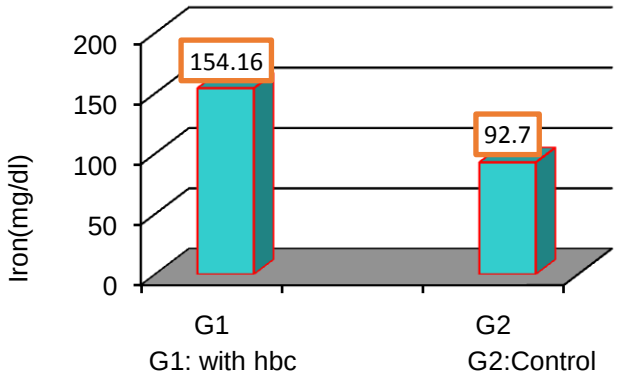


Figure (3). Compare between difference groups in Iron



Recent studies have indicated that measurement of ferritin, the major cellular storage protein for iron [29] may allow for such early detection of HCC. In vitro studies showed that iron induced increased ferritin synthesis by hepatoma cell lines and enhanced tumor cell growth Elevated serum ferritin was also associated with inflammation and liver diseases Moreover, ferritin that is synthesized by tumor cells may exert adverse effects on host immune responses and defense mechanisms Population studies have clearly documented that HCC patients have a higher level of ferritin than healthy subjects or patients with other liver diseases[25,30] but retrospective studies cannot clarify whether the increased level is the cause or consequence of HCC because ferritin is also produced by the tumor cells. Meanwhile, very few studies have prospectively evaluated the relationship between serum ferritin level and HCC risk in HBV patients [31] A study conducted in Tunis,Tunisia patients with chronic hepatitis B and cirrhosis reported that males with sustained high serum ferritin levels had a high chance of developing HCC [32].In CHC, serum ferritin can be elevated because of HCV induceddownregulation of hepcidin. Liu et al [33] proved that HCV can inhibithepcidin mRNA in Huh7.5 cell line followed by increased hepatic iron. Accumulated iron can lead to oxidative stress, hepatic fibrosis, and cirrhosis. They also reported that hepcidin reduced HCV replication in Huh7.5 cell

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line [34]. Increased iron can influence the HCV replication but is more likely to contribute to disease by potentiating oxidative stress which leads to chronic inflammation. Sumida et al5 described that there was a significant strong relation does exist between hepatic fibrosis and steatosis analyzed by linear modeling. The mechanism of this theory is steatosis that has positive correlation in lipid peroxidation and hepaticfibrosis. In fact, the elevation of marker of oxidative stress, serum **thioredoxin**, has an association with hepatic fibrosis and the serum lipid peroxide level in HCV-infected patients. [35] In our study, there was significant correlation between liver steatosis and hepatic fibrosis grade in univariate analysis but not in multivariate logistic regression analysis

Age & BMI

In this study, the results of patients with hepatitis B infection were compared with those of healthy subjects Table (2) and (Figure 4) showed results of NS: Non-Significant increase in age in G1 as compared with G3. Additionally, the result of BMI in Table (2) and Figure (5) revealed a Simple rise in G1 as compared G3

Table (2) Age and Body Max Index in two groups (patients and healthy)

Group	Mean ± SE	
	Age (year)	BMI (kg/m^2)
G1	43.37 ±2.47	28.05±0.66
G2	43.51 ±2.54	26.24±0.36
P-valueG1&G2	0.96 NS	0.018 NS

**** (P≤0.01), NS: Non-Significant.**

In this study, the age ranged from 18 years to 77 years, the initial study population included Forty- three patients were included, were infected with hepatitis B virus it also included Forty-three healthy people, with average age forpatients with hepatitis B, and control was (43.37±2.47, 43.51±2.54 year) respectively Which means that their ages are close, so there is no significant impact of age on the study.

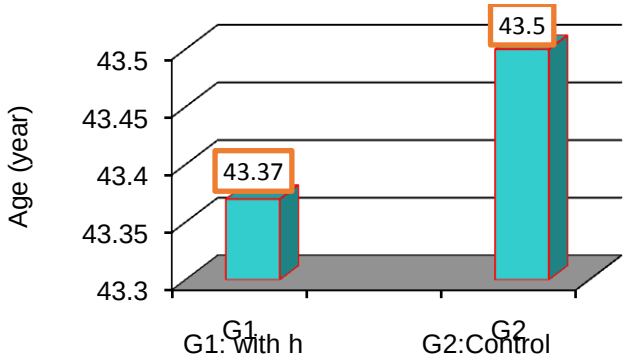

Figure 4. Compare between difference groups in Age

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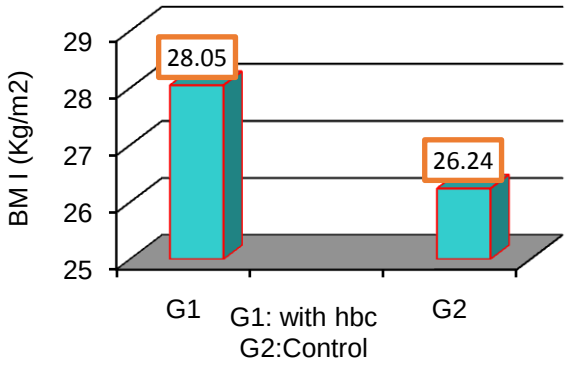
The current study has shown that Age was not an influential factor, however Several studies prove that ageing of hepatitis B and C Patients leads to increase in iron levels that occurs with aging in many tissues may exacerbate age-related diseases through iron-induced oxidative stress. Iron overload has been implicated in age-related neurodegenerative diseases affecting the brain (including Parkinson's and Alzheimer's diseases) and affecting the retina (age-related macular degeneration [AMD]). In AMD, iron accumulates in photoreceptors, RPE, and Bruch's membrane [36]. Another study was conducted on 85-year olds show that older persons with anemia of inflammation have higher hepcidin levels than their counterparts without anemia. [37].

Figure 5. Compare between difference groups in BDI



For HBV incidence and mortality, it has been identified large evidence gaps in all population groups It has been postulated that there is a very strong association between Hepcidin expression withe the High Body Mass Index, when defined as a BMI greater than 30 kg/m2, is a risk factor for nonresponse to antiviral therapy independent of genotype and the presence of cirrhosis on pretreatment liver biopsy in the patients independent (of genotype and cirrhosis) negative predictor of response to hepatitis treatment [38] body-mass index (BMI) is associated with liver-related morbidity and mortality among hepatitis B virus (HBV) carriers excess body weight is involved in the transition from healthy HBV carrier state to HCC and liver-related death among patient Using different cut points for BMI, a study performed in HBsAg carriers shows that excess weight increased risk for developing patients had a risk of death from liver-related disease almost two times as great as patients of normal weight. they also observed that high BMI was associated with elevated serum ALT and GGT activity, which are significant predictors of risk for incident HCC and liver-related death. the study conducted among people recognized by their prevalence of excess consumption of alcohol, an important cause of cirrhosis and HCC [39].

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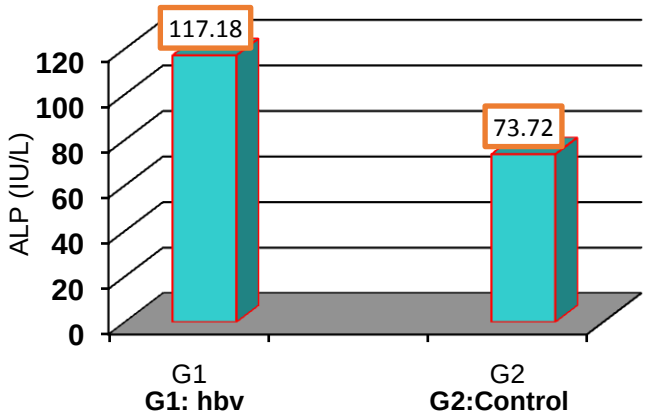
liver enzyme:alkaline phosphatase(ALP)

The pattern of liver enzyme changes is often the first piece of evidence that catches the doctor's attention**.** There was a high significantexcessof ALPdetected in Patient group as Compared to healthy individuals as reveled in Table (3) and figure (6). The high concentration of serum hepatic enzymes (ALP) may be associated to some extent with the elevated serum ferritin level, which in turn depends on the iron overload in hepatitis patients [40].ALP is an enzyme that transports metabolites across cell membranes. Liver and bone diseases are the most common causes of pathological elevation of ALP levels, although ALP may originate from other tissue, such as the placenta, kidneys or intestines, or from leukocytes [41] Hepatic ALP is present on the surface of bile duct epithelia. Cholestasis enhances the synthesis and release of ALP, and accumulating bile salts increase its release from the cell surface. ALP half-life in the circulation is about 1 week. These characteristics explain why ALP levels usually rise late in bile duct obstruction and decrease slowly after resolution [42]. In general, the blood parameters may affected by different factors inside or outside body lead to change in their levels such as X-ray [43].

Table (3) ALP Level in patients and control groups

	Mean ± SE
Group	ALP (IU/L)
G1	117.18 ±6.23
G	73.72 ±3.50
P-value G1&G2	**HS**
(P≤0.01).	

Figure 6. Compare between difference groups in ALP



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Correlations among Study Analysis

Table (4): Correlation coefficient (r) between serum hepcidin and other studied parameters.

Parameters		Correlation coefficient-r with Hep.(pg/ml)	
		G1	G2
Age	r.	0.15	0.13
	p.	HS	HS
BMI	r.	-0.17	-0.12
	p.	HS	HS
Ferritin	r.	0.37	-0.02
	p.	HS	HS
Iron	r.	-0.52	0.19
	p.	0.NS	0.HS
ALP	r.	-0.01	-0.37
	p.	HS	HS

Correlation of Serum hepcidin and Age

There was a positive correlation between serum hepcidin and age (r= 0.15, $p= 3.06\times10^{-54}$), (r=0.13$p = 8.48 \times 10^{-60}$) in G1, and G2 respectively, and it was significant as revealed in Table (4).

Correlation of Serum hepcidin and BMI

There was a negative correlation between serum hepcidin and BMI in G1, G2 (r = -0.17, $P = 3\times10^{-73}$), (r = -0.12, $P =1.3 \times10^{-54}$) respectively correlation as revealed in Table (4).

Correlation of Serum hepcidin and Ferritin

There was a positive correlation between serum hepcidin and Ferritin (r= 0.037, $p= 3.3\times10^{-50}$), in G1, but it was a negative correlation (r= -0.02 $p = 1.2 \times 10^{-11}$) in G2 and it was significant as revealed in Table (4).

Correlation of Serum hepcidin and Iron

There was a negative correlation between serum hepcidin and Iron (r= -0.52, $p= 0.28$), in G1, but it was a positive correlation (r= 0.19 $p= 8.8 \times10^{-28}$) in G and it was significant in G3 but it wasn't significant as revealed in Table (4).

Correlation of Serum hepcidin and ALP

There was a negative correlation between serum hepcidin and ALP in G1 and G2 (r = -0.01, $P = 7.05\times10^{-7}$), (r = -0.37, $P = 9.1 \times10^{-35}$) respectively as revealed in Table (4).

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