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Evaluation of the Level of Histamine 1 and 2 Receptors with Some Biochemical Variables in Patients with Hepatitis C Virus Infection

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ABSTRACT

The current study was carried out at Ibn - Sina Hospital in Nineveh Governorate, where the relationship between histamine receptors1&2, liver enzyme functions, Albumin, and Alkaline phosphate was studied in patients with hepatitis C virus type. Samples were taken from patients diagnosed with hepatitis C, 60 of whom were compared with 30 controls. Take 5ml of blood, separate it with a centrifuge, and test the serum. The sample size was equal for Men and Women, and the age range was 18 to 78 years. ALT (GPT), Albumin, Alkaline phosphates, and histamine-2 receptor levels were statistically significant, while AST (GOT) and histamine-1 receptor levels were not statistically significant.

Keywords- histamine receptor1 - histamine receptor2 - hepatitis C virus infection.

I. INTRODUCTION

Histamine is involved in a variety of physiological functions. The local action of histamine plays a role in providing metabolic energy to tissues. It is an important component and mediator of many biological processes. It has high potency even at low concentrations, and its concentration and secretion are tightly controlled to avoid unwanted reactions. Its biological effects have been described for over 100 years and have been shown to play a role in vasodilation and smooth muscle contraction in the bronchi, uterus, and intestine. It stimulates histamine by decarboxylation of the amino acid (L-histidine) in the cells that produce it, such as mast cells and basophils (Hattori & Seifert, 2017). Histamine receptors have been of interest in the therapeutic field. Some of these have been identified, e.g. (histamine receptor1) and (histamine receptor2) in allergy and sleep disorders are mainly expressed on gastric parietal cells, where suppressor T cells play a role in controlling their levels, making it an important target for the treatment of gastric ulcers (Tiligada *et al*., 2017). Due to the risk of liver cancer, the body plays a protective role in the production and activation of histamine in the body, as it is a bioactive substance that stimulates the body's inflammatory and immune responses by affecting mast cells and becoming the target of the treatments used have become. Histamine 1 receptors also play an active role in allergic lesions with anti-inflammatory properties. Used for reproduction, may be effective in cancer treatment by controlling some cancers (Shen *et al*., 2022). Evidence has accumulated over time regarding the role of histamine 2 in renal dynamics and its role in renal pathophysiology, as the kidney synthesizes and maintains histamine with its receptors, allowing histamine Widespread roles in the kidney Consistent hemodynamics Complementary roles of renal filtration and reabsorption play an important role in therapeutic response (Grange *et al*., 2020).

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease and associated morbidity and mortality worldwide (Chigbu et al., 2019). Short-term, oral, curative, direct-acting antiviral therapy has transformed the treatment of HCV infection. Since the launch in 2016 of the first global strategy to eliminate viral hepatitis as a public health threat by 2030 (Indolfi

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et al., 2019) . Viral hepatitis (C) was a human problem until scientists were able to discover it, when the Sherwin-Williams Institute and the CDC announced in 1989 that scientists Chose discovered it using polymerase chain reaction (PCR) technology instead of an electron microscope Becoming other viruses, formerly known as hepatitis (Non-A - Non-B), is viral hepatitis caused by viruses other than (A) or (B) viruses. Hepatitis viruses are considered to be relatively small (40-60) nm in diameter and belong to the family Flaviviridae, a spherical virus with an envelope composed of three main components: viral envelope, envelope, and single-stranded RNA. The last two These components make up the so-called virus core(Hatzakis, et al (2021)).

HCV test: The Enzyme-Linked Immunosorbent Assay (Enzyme-Linked Immunosorbent Assay) method was used to search for antibodies to hepatitis C virus (Anti-HCV Ab) (IgG, IgM) in the indirect way in serum or plasma, where the antibodies in the sample bind with the antibody in the pits ELISA strips Parameter (Ag) is conjugated by the Horseradish peroxidase enzyme (HRP-Protein A Conjugate), where an immune enzyme complex is formed that can be detected by adding a substrate solution (TMB - Substrate) to the etching of the ELISA strips to give a blue color, and then it is stopped. The reaction by adding a stop solution (sulfuric acid 1N). The chromatic density (O.D.) was read by the special reader of the ELISA system at the wavelength (450/620-690 nm). Where the intensity of the concentration of antibodies in the sample is directly proportional to the color intensity (Wilber.J.C).

II. MATERIALS AND METHODS

1. Sample collection: From December 1, 2022, to February 30, 2023 Samples were taken from Ibn - Sina Hospital for patients and control samples, where the total number was 90 samples divided into Group A 60 patients the number of Males and Females was equal were (30 males and 30 females) and Group B 30 controls (15 males and 15 females). The samples were divided into three groups, the first being patients and control, the second according to sex, and the third according to age groups Samples collected from patients diagnosed with hepatitis C virus Blood by drawn 5 ml from the patient with a new sterile syringe, and each sample was placed in a glass test tube with a sealed rubber cap filled with solidified gel and left at room temperature for two hours. Blood samples were then centrifuged at 1,000 g for 20 minutes and serum was stored at -20°C.

2. Methods:

1. Histamine 1 and 2 receptors:

The working principle is based on the use of the quantitative immunoassay method using the sandwich ELISA technique, where the antigen that will be detected is incubated with antibodies added during the working method present in the test box. The test pits are coated with antibodies specific to the interleukin to be searched for and then the samples for the research are added, followed by the addition of the biotin antibody for the interleukin after getting rid of any unrelated compounds, then the Horseradish peroxidase enzyme (HRP-Protein A Conjugate) is added after washing the pits. (The sulfuric acid is 1N) to turn the color into yellow, the intensity of the color is directly proportional to the amount of antibodies in the sample, then it is read at the wavelength (450 nm) (Human HRH1 (Histamine H1 Receptor) ELISA Kit, n.d.; Human HRH2 (Histamine H2 Receptor) ELISA Kit, n.d.).

2. Body mass index (BMI):

Body mass index is a value derived from a person's weight and height. BMI was defined as body weight divided by the square of body height and measured in (kg/m^2) (Di Angelantonio *et al*., 2016).

3. Biochemical test:

- **ALT/GPT – AST/GOT:**

The method of quantitative determination of the enzyme was used in the research samples, where the enzyme (ALT) catalyzes the transfer of the amino group from (L-alanine) to (α-ketoglutarate), which leads to the formation of (pyruvate) and (L-glutamate) (Scherwin, J.E, Liver)

$$L-Alanine+\ \alpha-Ketoglutarate \xrightarrow{ALT} Pyruvate+L-Glutamate$$

Lactate dehydrogenase catalyzes the reduction of pyruvate and the simultaneous oxidation of (NADH) to (NAD). The rate of the resulting decrease in absorption is directly proportional to the activity of (ALT) when the reagent is formed during the reaction.

$$Pyruvate+\ NADH+H^+ \xrightarrow{LDH} L-Lactate+NAD^+ +H2O$$

In the examination of the AST enzyme, the method of quantitative determination of the enzyme was used in the research samples, where the AST enzyme catalyzes the transfer of the amino group from (L - Aspartate) to (α - Ketoglutarate), which leads to the formation of (Pyruvate) and (Oxalacetate) (Scherwin, J.E, Liver).

$$L-Aspartate+\ \alpha-Ketoglutarate \xrightarrow{AST} Oxalacetate+L-Glutamate$$

Oxalacetate undergoes reduction with simultaneous oxidation of NADH to NAD, in a malate-catalyzed dehydrogenation reaction. The resulting rate of decrease in absorbance is directly proportional to AST. LDH is added to prevent interference from endogenous pyruvate, which is normally found in serum.

$$Oxalacetate+\ NADH+H^+ \xrightarrow{MDH} L-Malate+NAD^+ +H2O$$

- **Alkaline Phosphatase:**

Alkaline phosphate is determined in blood serum by measuring the rate of hydrolysis of phosphate ester. Alkaline phosphate stimulates the hydrolysis of nitrophilic phosphate at a basic temperature (10.4),

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which leads to the release of nitrophenol and phosphate according to the following equation (Wenger C. et al.).

$$p-Nitrophenylphosphate + H_2O \xrightarrow{Alk.Phos.} Phosphate + p-Nitrophenol$$

- **Albumin:**

At a controlled pH, the bromcresol is a green compound with albumin. The intensity of the color is directly proportional to the albumin content. The initial absorption is obtained as suggested by Webster and according to the following equation (Webster D).

$$BCG + ALBUMIN \xrightarrow{controlled\ pH} Green\ BCG/ALBUMIN\ Complex$$

3- Statistical analysis:

This was done using SPSS software (Statistics Packages for the Social Sciences) (version 26) and Microsoft Office Excel (2016). The data were analyzed to determine the difference between the study groups using a one-way analysis of variance (ANOVA). A correlation test was used to examine the correlation between the parameters studied. Results are presented as mean standard deviation (mean ± SD). Statistical significance at levels (p 0.01) is (p 0.05).

4- Results and Discussion: Human and biochemical parameters for all participants were as shown in Tables:
A – Patients and controls:

Table (1) Comparison between laboratory data of controls and patients

parameters	Control mean ± SD	Patients mean ± SD	p-value
Age	37.13 ± 8.45	44.8 ± 16.83	0.858
HRH1 ng/mL	3.250±0.542	3.334±0.943	0.452
HRH2 ng/mL	8.148±1.218	9.080 ±1.905	0.035
AST/GOT IU/L	10.47±2.33	12.24±5.54	0.227
ALT/GPT IU/L	8.563±3.142	10.627±4.670	0.039
Albumin g/dL	41.33±4.32	34.218±5.52	0.000
Alkaline Ph. IU/L	180.80±49.29	209.875±86.001	0.000

In current study, the level of histamine 1 receptor (Figure (1)) in patients was higher than that of control samples, but without a statistical significance between the ratios. The final stage and those undergoing dialysis compared to control are higher (Grange *et al*., 2020). A study (Sudarikova *et al*., 2021) is similar to the current study, in which it was mentioned that the role of the histamine 1 receptor extends to modifying kidney function. In our study, patients suffer from kidney failure and depend on hemodialysis in their lives as a solution Compensatory for the damage in the kidneys, and histamine has an active role in the vessels and renal infections, as well as its prominent role in liver injuries as well, as it enhances blood flow to the inflamed tissues as a result of the expansion of blood vessels and increased capillary permeability, thus recruiting many white blood cells at the site of inflammation and fighting the foreign body.

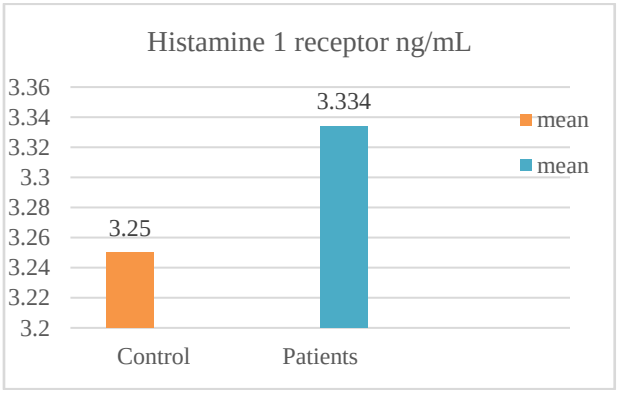

Figure 1: Histamine 1 receptor

In similar studies that showed an increase in the level of histamine receptor 2 (Figure (2)) in patient samples, the study of Shan and his group (Shan et al., 2019) is similar to our current study. By secreting the histamine receptor 2 because it is one of the inflammatory cytokines and is a regulator of programmed cell death and the release of inflammatory peptides that help the migration of defensive cells to the affected areas. (Vanina Medina *et al*., 2020) mentioned in his study that histamine produced dual effects on the growth of pathological cells, which reduced the proliferation of fibrotic cells in the liver. Basophils mature and are released into the blood, while mast cells mature from the bone marrow, which arise from myeloid precursors. Which usually mature in tissues, and the maturation leads to the release of many inflammatory mediators, including the histamine 2 receptor, which increases the decomposition of infected cells, which leads to an increase in its blood level. Therefore, this study was also similar to current study.

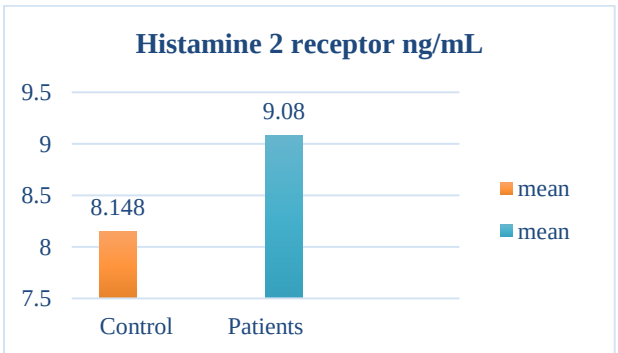

Figure 2: Histamine 2 receptor

The figure (3) showed that there was no significant difference for the AST/GOT enzyme between

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patients and healthy people, as the study showed that there was no increase in the enzyme value in patients with viral hepatitis C, because the hepatitis did not turn into advanced stages such as cirrhosis and it is still in the early stages. Similar to the study by Rogachev (Rogachev *et al*., 2012) and Amjad (Amjad *et al*., n.d 2021.).In addition to liver injuries, the enzyme elevation can be caused by strenuous exercise, the use of muscle-building supplements, or weight loss. Its elevation has also been associated with the use of anabolic steroids, and here the elevation of the enzyme is not useful in distinguishing between liver and muscle injury, as stated in the study conducted by Kim and his group (Kim & Wu, 2020). Also, one of the studies conducted in Khorasan Province in Iran, carried out by Nakhaee indicated that liver injuries result from exposure to lead and are the result of the enzyme Aspartate Aminotransferase normal within normal levels in blood serum (Nakhaee *et al*., 2019). The above studies agreed with the results of our current study.

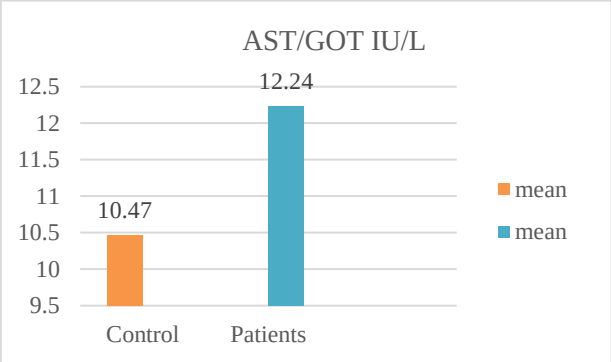

Figure 3: AST/GOT

A significant difference appeared with an increase in the ALT/GPT enzyme rate between patients and healthy people Figure (4), as the enzyme rate increased in people who suffer from problems in the liver, kidneys, muscles and heart compared to healthy people, and this result agrees with a study (Yuk *et al*., 2019), in which it was shown that the enzyme rate increased in patients with hepatitis and kidney problems and the urinary system. Also, as a result of liver and kidney disorders, people suffer from high blood pressure, diabetes, and osteoporosis, which has a role in the increase in the enzyme level in patient samples, according to a study (Choudhary *et al*., 2021).

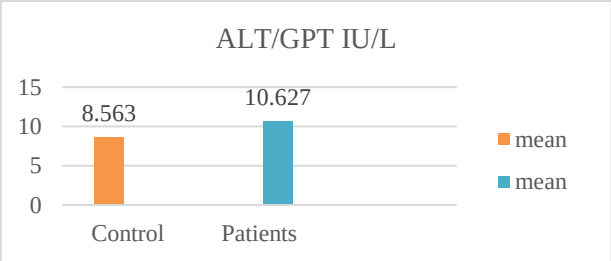

Figure 4: **ALT/GPT**

Serum albumin Figure (5) is the most abundant protein that has a range of functions including molecular transport of substances, antioxidants, anti-inflammatory, modulation of capillary permeability (Sun *et al*., 2019) and regulation of various body functions, but in patients with liver problems its levels are It is lower than healthy people due to disease and therapeutic interventions, and this is what came in agreement in this study with the study of Rakesh Kumar and his group (Jagdish *et al*., 2021), where his study stressed the need to give intravenous albumin solutions to patients who suffer from hepatitis and kidney failure and are constantly on hemodialysis. As mentioned, (Elloumi *et al*., 2019) on giving albumin to patients at a dose of (30 gm / day) an improvement in the condition of patients was noted between the first and third day, and it reduced the rate of cirrhosis of the liver and the deterioration of the condition of the kidneys.

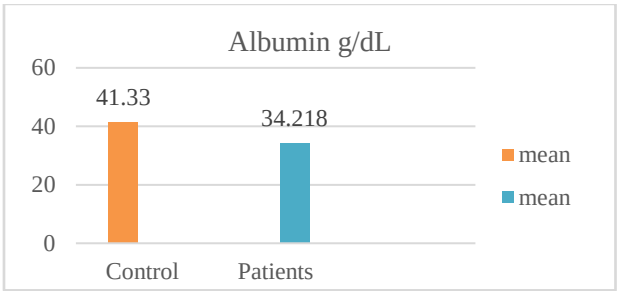

Figure 5: Albumin

Alkaline phosphates Figure (6) it is an enzyme that plays important roles in various functions such as the hydrolysis of a certain group of physiological compounds containing phosphates and contributes to the synthesis of DNA and bone calcification, and any disturbances in the secretion of the enzyme lead to serious pathological conditions (Zaher *et al*., 2020). The results of the current study agree with the results of the study of (Q. Li *et al*., 2020) and his group, where the high level of enzyme in the blood serum was considered evidence of problems in the liver or kidney, despite its association with bone injuries as well. The results of the current study were also similar to a study in Sudan on patients with chronic renal failure, which was conducted by (Modawe *et al*., 2021) and his group, where the enzyme ratio increased in patients with renal failure compared to healthy subjects of the study samples.

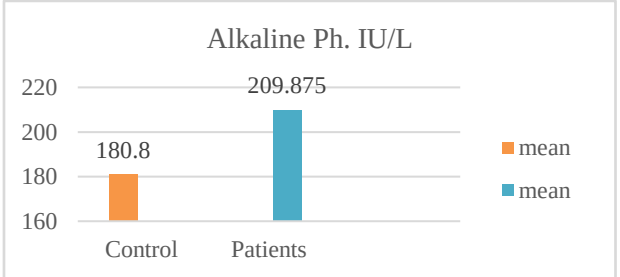

Figure 6: Alkaline Ph.

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B - Males and Females:

Table 2: Comparison between laboratory data of Males and Females

parameters	Males mean ± SD	Females mean ± SD	p-value
Age	43.83±6.35	46.4±5.47	0.32
HRH1 ng/mL	2.976±0.983	3.625±0.884	0.007
HRH2 ng/mL	8.849±1.298	9.234±2.026	0.401
AST/GOT IU/L	12.30±4.64	12.05±5.19	0.828
ALT/GPT IU/L	11.267±5.04	9.776±4.430	0.202
Albumin g/dL	34.58±5.71	33.86±5.39	0.577
Alkaline Ph. IU/L	205.95±90.362	213.80±82.76	0.749

With regard to the effect of the level of histamine receptor 1 on gender (figure 8), the current study showed a significant difference between the sexes, as the percentage of histamine receptors in females was higher than in males. Our study was similar to the study of Yoshizawa and his group (Yoshizawa *et al*., 2009) and the study of Takanami (Takanami. *et al*., 2021). Female hormones have a major role in the increase in the proportion of histamine receptors compared to males, and among these hormones is estrogen, which explained the reason for reporting that females are more sensitive to pain than males, and (Chen *et al*., 2008) mentioned in his study that the percentage of histamine receptor 1 decreases in females after Menopause due to the difference in the proportions of sex hormones.

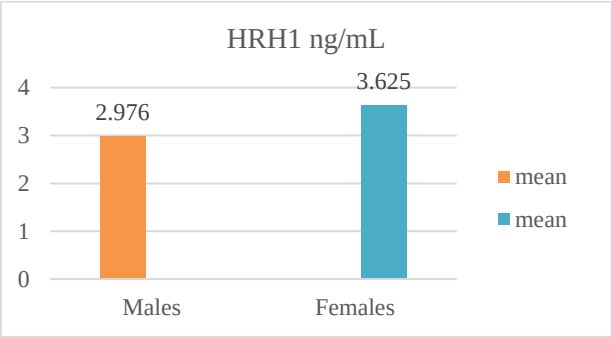

Figure 7: Histamine receptor 1

As for the level of histamine receptor 2 being affected by gender (figure 9), its rate was higher in females than in males, but there was no significant difference, due to female sex hormones and the difference in their ratios during the month in the phases of the menstrual cycle. These results were similar to the study of (Ridolo *et al*., 2019) and the study of (Chiarella *et al*., 2019). al., 2021) These hormones affect the increase in the number of innate lymphoid cells, unlike the male hormones that negatively affect them.

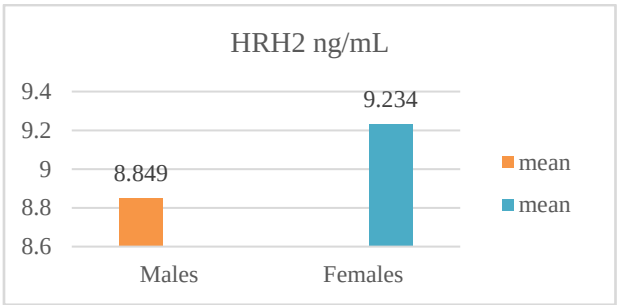

Figure 8: Histamine receptor 2

There is no significant difference in AST/GOT enzyme elevation between males and females according to Rogachev (Rogachev *et al*., 2012). (Figure 10).

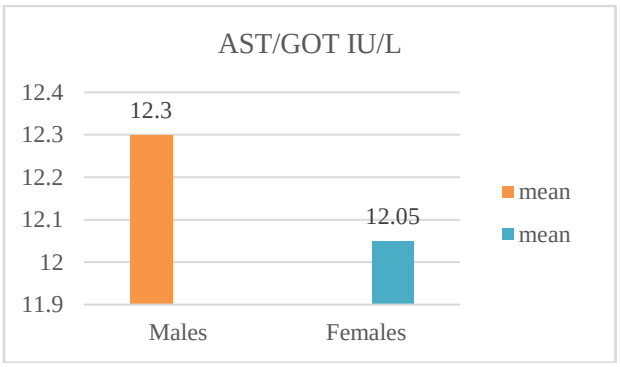

Figure 9: AST/GOT

The study of (J. Zhang *et al*., 2020) was similar to study in terms of the enzyme elevation rate between males and females (figure 11), which are close without a significant difference between the groups, as was shown in current study.

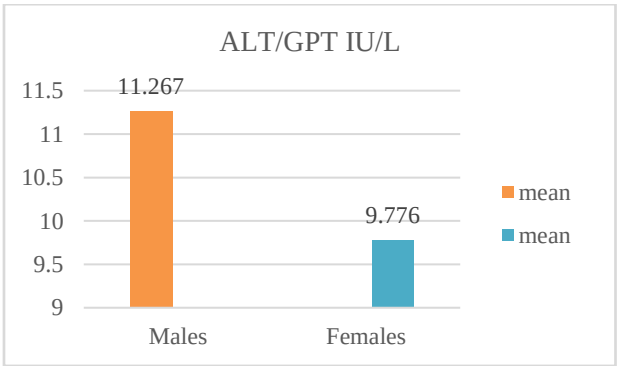

Figure 10: ALT/GPT

As for the association of the two albums with gender (figure 12), there is no significant difference in current study, and this is similar to the study (Castellazzi *et al*., 2020), where the study showed that there was no significant difference in the ratio of the two albums by gender.

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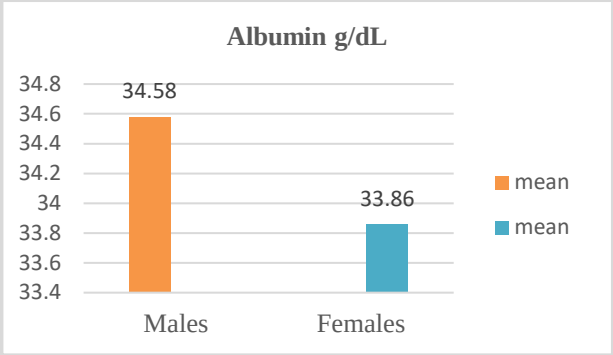

Figure 11: Albumin

For males and females Figure (13), the sex hormones control the Alkaline Phosphatase enzyme ratio inside the body, so there is no effect of gender on the enzyme ratio, as the sex hormones have a role in building bones and maintaining the alkaline phosphatase enzyme ratio within the normal limits, but it differs in transgender people due to the difference in the ratio of hormones inside the body because of their different levels for therapeutic reasons According to the study conducted by (Vlot *et al*., 2019).

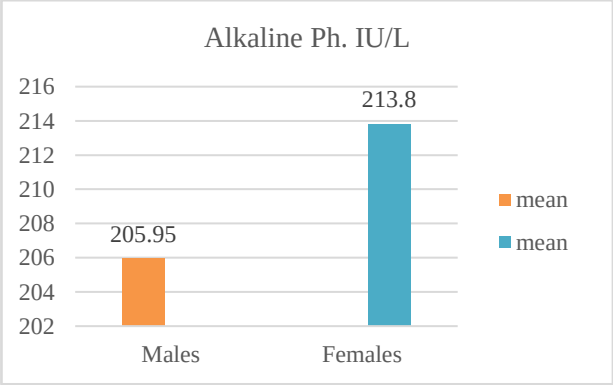

Figure 12: Alkaline Ph

C – BMI and other Parameter:

Table 3: Comparison between laboratory data of BMI and other Parameter

Parameters	BMI < 25mean ± SD	BMI ≥ 25mean ± SD	p-value
HRH1 ng/mL	3.440±0.971	3.079±1.068	0. 020
HRH2 ng/mL	9.508±1.847	8.377±1.319	0.012
AST/GOT IU/L	12.571±5.023	11.785±4.961	0.580
ALT/GPT IU/L	11.627±5.104	9.730±4.002	0.161
Albumin g/dL	34.765±5.026	34.365±6.738	0.850
Alkaline Ph. IU/L	219.544±84.013	200.150±93.578	0.436

There is a direct relationship between the increase in the level of histamine receptors 1 with the body mass index, as it increases in people who have a higher body mass index Perhaps the weight observed with prescription H1 antihistamine use is related to the disruption of H1 receptor expression and binding leading to impaired insulin and leptin signaling. More research is necessary to determine the extent of histamine's role in energy metabolism. (Ratliff et al., 2010).

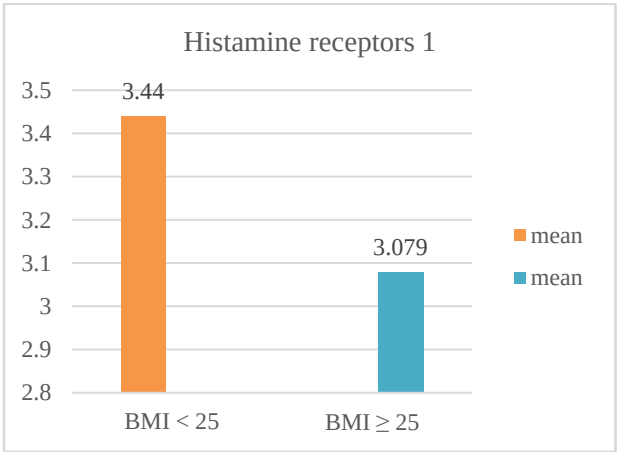

Figure 13: Histamine receptors 1

The suppressive and selective effect of histamine on visceral fat deposition likely depends on activation of the sympathetic nervous system then There is a direct relationship between the increase in the level of histamine receptors 2 with the BMI (Yoshimoto et al., 2006) . Histamine also affects peripheral metabolism by increasing lipolysis in white adipose tissue (Kotańska et al., 2018).

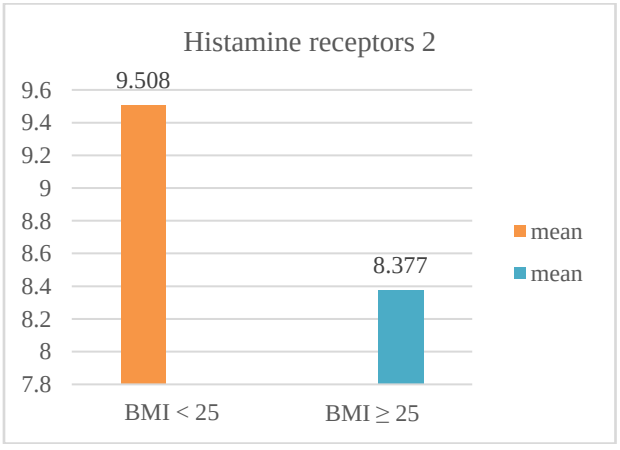

Figure 14: Histamine receptors 2

There is no statistical significance between BMI and GPT and GOT enzymes because they are related to liver disease and not BMI (*Mukai2002*, n.d.) . Also, treatment of viral hepatitis has a role in reducing the ratio of the two enzymes and is not related to the body mass index (Batsaikhan et al., 2018) .

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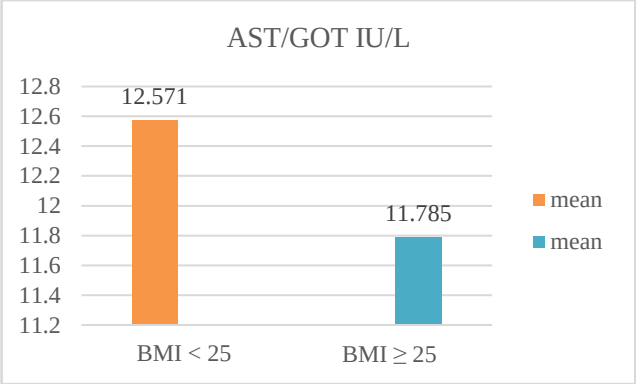

Figure 15: AST/GOT

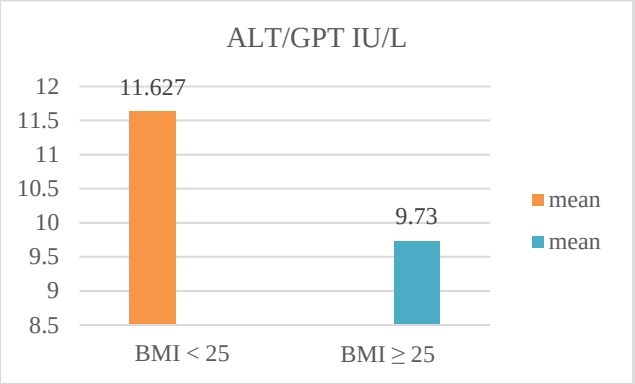

Figure 16: ALT/GPT

The result of albumin protein in patients with hepatitis who had a body mass index less than 25 was higher than those whose body mass average was greater than 25, but without statistical significance, and the study matched (Ghany et al., 2010).

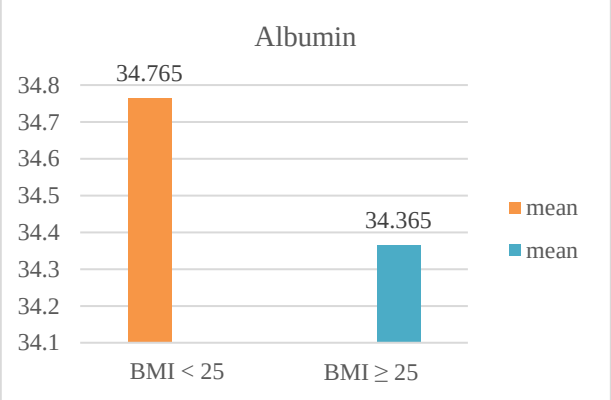

Figure 17: Albumin

There is no statistical significance in the result of alkaline phosphatase between the two groups, and the study matched the study (*Hamoui2004*, n.d.) and (Gürler & Çeçen, 2020) elevation in ALP levels is not associated with BMI. It might be a response to elevated parathormone levels for a defect in liver function by activating vitamin D3 and the fact that infectious alkaline is also excreted from the bones (Rahman & Branch, n.d.).

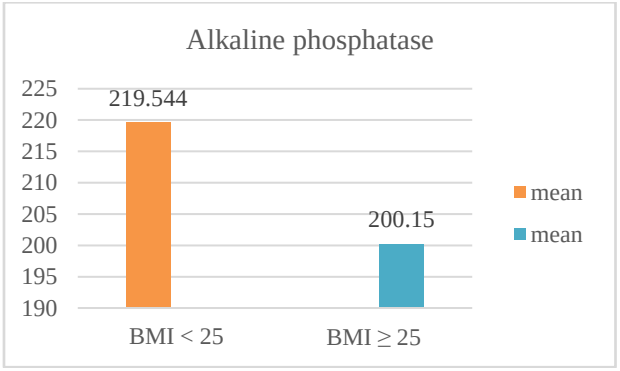

Figure 18: Alkaline phosphatase

Correlations:

Table 3: Correlations between Parameter

Parameter	Correlations	H2r	H1r	GOT	GPT	Alkaline Phosphatase	Albumin	BMI
H2r ng/mL	Person cor.	1						
	P value	---						
H1r ng/mL	Person cor.	0.489*	1					
	P value	0.000	---					
GOT IU/L	Person cor.	0.042	0.055	1				
	P value	0.748	0.678	---				
GPT IU/L	Person cor.	0.101	0.079	0.684*	1			
	P value	0.445	0.550	0.000	---			
Alkaline Phosphatase IU/L	Person cor.	0.005	0.110	0.138	0.204	1		
	P value	0.968	0.405	0.291	0.117	---		

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Albumin g/dL	Person cor.	0.204	0.176	0.158	0.265*	0.059	1	
	P value	0.117	0.180	0.227	0.041	0.654	---	
BMI kg/m2	Person cor.	-0.264*	-0.089	-0.057	-0.052	0.149	0.084	1
	P value	0.041	0.498	0.667	0.694	0.256	0.522	---

- **Correlations between Histamine receptor 1 and Histamine receptor 2:**

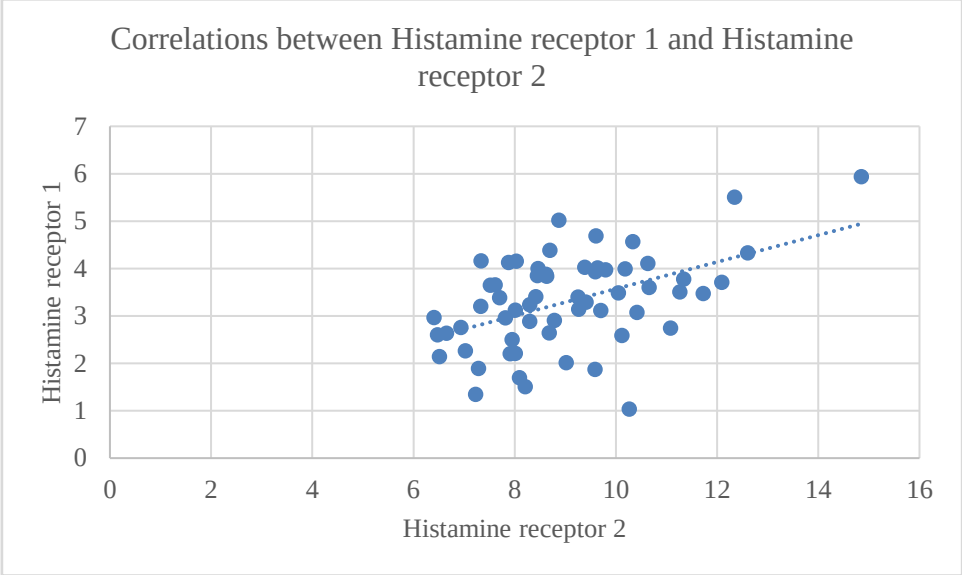

Figure 15: Correlations between Histamine receptor 2 and Histamine receptor 1

There is a positive significant correlation was noted between Histamine receptor 2 and Histamine receptor 1, because histamine is a biologically active substance that stimulates the inflammatory and immune response of the body (Shen *et al*., 2022).

- **Correlations between (AST/GOT) and (ALT/GPT):**

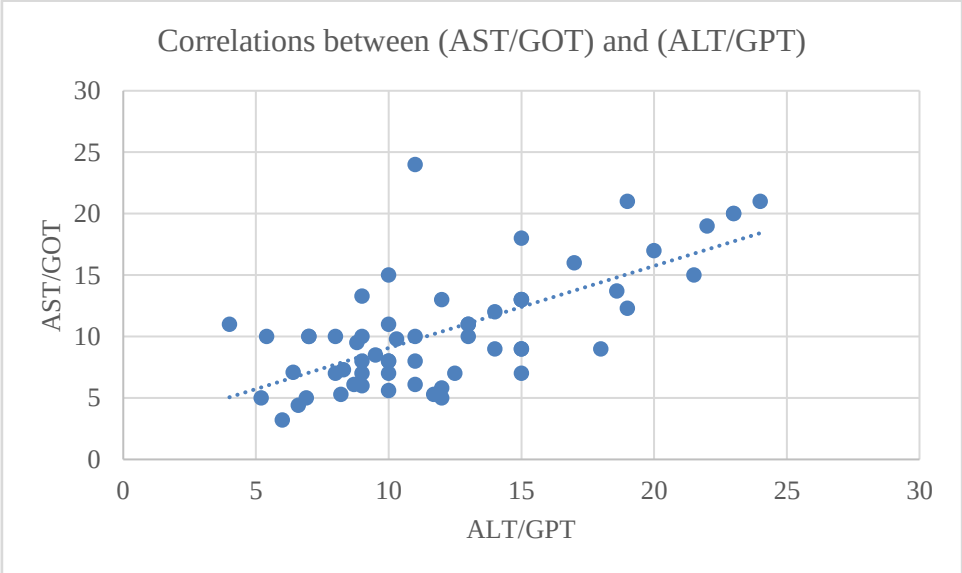

Figure 16: Correlations between (AST/GOT) and (ALT/GPT)

There is a positive correlation between the two and these matches (Kanjanadecha & Sethasine, 2019) where values are elevated due to liver injury.

- **Correlations between Albumin and (ALT/GPT):**

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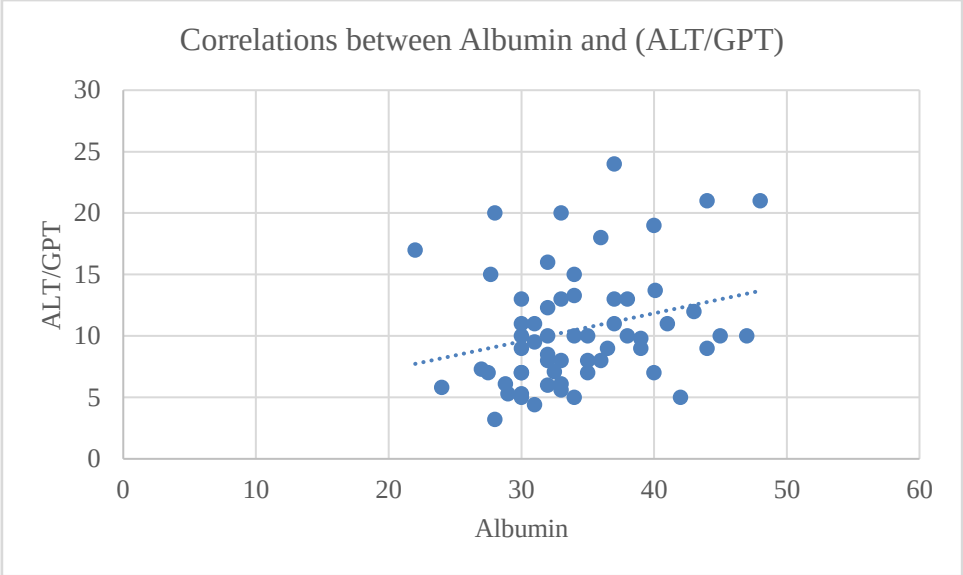
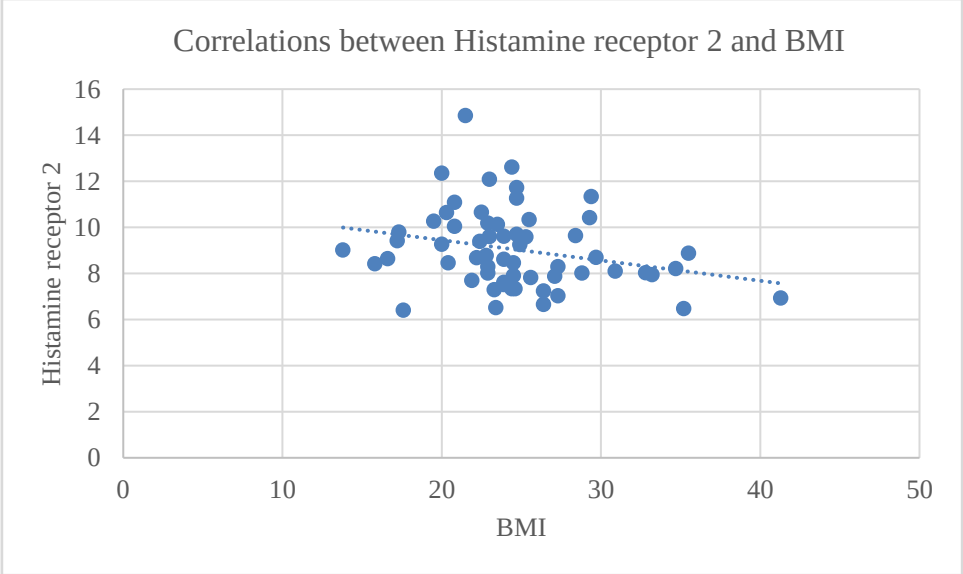


Figure 17: Correlations between Albumin and (ALT/GPT)

There is a positive correlation between Albumin and (ALT/GPT) and these matches (Alsalih et al., 2021) where values are elevated in acute or long-term liver disease.

- **Correlations between Histamine receptor 2 and BMI:**



There is a negative relationship between the two because Histamine also affects peripheral metabolism by increasing lipolysis in white adipose tissue (Kotańska et al., 2018) .

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