

## Study of Several Important Immune Markers in Hepatitis B patients

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### **Abstract:**

Hepatitis B patients have been collected from Marjan Teaching Hospital in Babylon Province through the period from May/2017 to September/2017. Were for study of several immune markers in serum isolated from these patients. The immune markers were involved of the antibody types as well as Rheumatoid factor and C-Reactive protein. The antibodies were involved IgA, IgM and IgG. The results were showed significant elevation in concentration of the IgG antibody and also elevation in concentrations of the IgM and IgA antibodies but no significant when comparison of these antibodies with apparently healthy persons antibodies concentrations. Also this study was explained increases in presence percentages of both the Rheumatoid factor and C-Reactive protein in serum of these patients in comparison with same apparently healthy persons that have no presence percentages for both Rheumatoid factor and C-Reactive protein.

**Key words:** Hepatitis B Patients, Antibodies, Rheumatoid Factor, C-Reactive Protein.

### **Introduction:**

The Virus of Hepatitis B is a type of the noncytopathogenic, hepato-pathogenic of the Hepadnaviridae family, that causes variable degrees of the humans liver diseases [1]. This virus can causes either chronic or acute infections, the infections in adult have relatively low chronicity rate about 5% and infections of neonatal mostly have high rate of persistence [2]. Chronic infections are usually asymptomatic, but carriers of this virus can be developing life threatening infections like cirrhosis and hepatic carcinoma [3]. Despite the

availability of the prophylactic vaccines, hepatitis B virus is estimated to infect around 400 million peoples worldwide, with endemic areas in Africa and Asia [1]. And the range of the infections in different countries from (0.1% to 20%) [4].

The response of the antibodies to the antigens of the enveloped viruses (like, HBV) play the specific roles in the removing of the these viruses from the bloodstream of infected patients, and this acting for decreasing the releasing of the viruses within the host, as well as in the protection against perinatal viral infections [5]. Titers detection of the total anti

Hepatitis B viruses in the serum mostly used as standard to response of the immunity against surfaces antigens of the hepatitis B virus [6]. Several studies have certain that the humoral immunity through neutralize antibodies response is more effective against many viral protein antigens [7].

The antibodies response to the virus of the Hepatitis B infections is difficult to experimentally study [8]. Free antibodies to the surface antigens is not detected until after the resolution of this virus infections [9]. The present of the antibodies associated with Hepatitis B viral infection in immune complexes in the circulating in both infections of the acute and chronic types, proposing that production of the antibodies are much speedily than identified and that they plays specific roles in the pathological disease [10]. The specific antibodies against the hepatitis B virus antigens have properties of neutralizing and mediate protective immune response [11].

The Rheumatoid factor; RF is a class of the antibodies that have several affinities and isotypes, were first reveal before more than seventy years ago, but there is yet much to discover about the mechanisms implied their production, pathological effects and physiological roles [12]. This autoantibody found directed against the Fc portion of the antibody (IgG) [13].

This factors have been reported that their appearance in the serum is sequential before diagnosis; first IgM RF, then IgA RF and

finally IgG RF [14]. It is found in the patients serum with diseases types, including autoimmune and nonautoimmune conditions [15]. And this factor associated with hepatitis B virus infections [16].

The C-reactive protein; CRP firstly identified and explained in 1930 by the Tillet and Francis and routinely considered an important immune regulator of the innate immunity [17]. They are the acute phase proteins, nonspecific industries by the liver in responses to the inflammations of the acute and chronic infections [18].

The production of the C-reactive protein is regulated through the cytokines of the proinflammatory type [19]. The studies demonstrated the production of this protein in correlates with progression diseases in chronic hepatitis B infections [20].

### **Materials and Methods:**

#### **The Patients and Samples**

The study cases were males and female of patients infected with Hepatitis B virus, they collected from Marjan Teaching Hospital in Babylon Province, the samples were serum taken from these patients and used for the study of the several immune markers concentration, the immune markers were IgA, IgM, IgG, RF and CRP.

**Identification of the Patients by (HBsAg EIA Test Kit Package Insert): according to the (ACON Laboratories Inc. San Diego; USA)**

The Patients were identified in hospital as Hepatitis-B-Patients according to (*Foresight*) an enzyme immunoassay (EIA) for the qualitative detection of the hepatitis B surface Antigen (HBsAg) in the human serum: this for the professional diagnosis use only [21].

**Determination of the antibodies (IgA, IgM and IgG) by the Radial Immunodiffusion.**

This according to (Roseto degli Abruzzi (Te) Italy, LIOFILCHEM(R) s.r.i.) was include:

1. Removed EASY RID from the envelope, open the plate and leave about (5 min) at room temperature so that any condensed water in the well can evaporate.
2. Filled the wells with the (5 µl) of undiluted patients samples (serums).
3. Closed the plate with the lid, after the sample have been diffusion into the gel, leave to stand, overturned into the envelope at room temperature for (48 h).
4. Measured the diameters of the precipitin rings using suitable devices.
5. Determined the concentrations of the antibodies were by comparison of the

formed rings with the attachment tables of the same company.

**Determination of the (Rheumatoid factor and C-Reactive protein)**

❖ **Rheumatoid factor: RF-Latex.**

This according to (SPINRECT,S.A Ctra.Santa Colma, 7E-17176 SANT) (ESTEVE DE BAS (GI) SPAIN).

❖ **C-Reactive protein: CRP-Latex.**

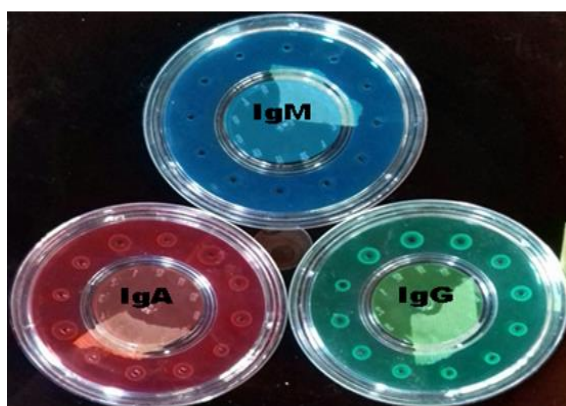
This according to (LINER CHEMICALS S.L. Joaquim Costa 18 2<sup>a</sup> planta. 08390 Montgat, Barcelona, SPAIN).

And determination of both the RF-Latex and CRP-Latex by Qualitative method (slide agglutination test) was include:

1. Left of the kit components and samples at room temperature, if the sensitive of this test decrease at low temperatures.
2. Placed a drop of sample on the custom circle in strip test.
3. Placed a drop of (RF or CRP) Latex reagent gently and next to the specimen drop.
4. Mixed well and then spread along the inner surface of the circuit in the test strip.
5. Slide the slide tilted forward and backward and continue for two minutes, with the positive result appearing in a agglutination manner.

## Statistical analysis

The results were analyzed by the Statistical Package Social Sciences (SPSS) [22], version 20 this for determination of the Mean, Medium, Standard deviation and Standard error, as well as the significant presence between antibodies have been calculated according to One Way ANOVA by descriptive excluded cases analysis by analysis with LSD at (95%) confidence and level of significant ( $P=0.05$ ).



**Figuer:1.** Zones were formed in the antibodies (IgM), (IgA) and (IgG) kits after two days from placed hepatitis-B-patients serum.

Figure (1) the zone were formed by reaction between the IgA, IgM and IgG antibodies kits with hepatitis-B-patients serum after (48 h) from placed the patients serum in the kits wells of these antibodies.

## Results:

The results explained elevation in concentrations of the antibodies (IgA, IgM and IgG) as well as presence percentage of the Rheumatoid factor and C-Reactive protein of

the patients when comparison with apparently healthy persons and these illustrated in following tables and figures.

**Table:1.** Concentrations of the antibodies types in apparently healthy persons.

Study groups	Statistical analysis	IgA (Mg/dL)	IgM (Mg/dL)	IgG (Mg/dL)
All apparently healthy persons	Mean	162.101	98.983	1184.834
	Standard Deviation	45.786	55.009	388.845
	Standard Error	13.218	15.879	112.249
Males apparently healthy persons	Mean	164.167	111.001	1214.334
	Standard Deviation	54.441	68.354	460.649
	Standard Error	22.226	27.905	188.059
Females apparently healthy persons	Mean	160.034	86.967	1155.334
	Standard Deviation	40.471	40.474	344.027
	Standard Error	16.522	16.524	140.449
*References normal Igs concentrations	IgA	90 – 490 (Mg/dL)		
	IgM	50 – 370 (Mg/dL)		
	IgG	800 – 1800 (Mg/dL)		
* The manufacture company of the antibodies kit				

Table (1) means of the IgA in all apparently healthy persons, males apparently healthy persons and females apparently healthy persons were 162.101 Mg/dL, 164.167 Mg/dL and 160.034 Mg/dL respectively; while for IgM were 98.983 Mg/dL, 111.001 Mg/dL and 86.967 Mg/dL respectively; and for IgG were 1184.834 Mg/dL, 1214.334 Mg/dL and 1155.334 Mg/dL respectively.

**Table:2. Concentrations of the antibodies types in Hepatitis B Patients.**

Study groups	Statistical analysis	IgA (Mg/dL)	IgM (Mg/dL)	IgG (Mg/dL)
All patients	Mean	348.791	177.362	2050.166
	Standard Deviation	143.455	73.469	738.298
	Standard Error of Mean	29.282	14.996	150.704
Males patients	Mean	337.667	128.726	1863.834
	Standard Deviation	143.857	61.813	866.468
	Standard Error of Mean	41.528	17.844	250.128
Females patients	Mean	359.917	226.001	2236.501
	Standard Deviation	148.544	48.001	559.648
	Standard Error of Mean	42.881	13.857	161.557
*References normal Igs concentrations	IgA	90 – 490 (Mg/dL)		
	IgM	50 – 370 (Mg/dL)		
	IgG	800 – 1800 (Mg/dL)		
*The manufacture company of the antibodies kits				

Table (2) means of the IgA in all Hepatitis-B-Patients, males Hepatitis-B-Patients and females hepatitis-B-patients were 348.791 Mg/dL, 337.667 Mg/dL and 359.917 Mg/dL respectively; whereas the IgM were 177.362 Mg/dL, 128.726 Mg/dL and 226.001 Mg/dL respectively; and the IgG were 2050.166 Mg/dL, 2236.501Mg/dL and Mg/dL 1155.334 respectively.

**Table:3. Significant differences between of the study groups according to LSD system.**

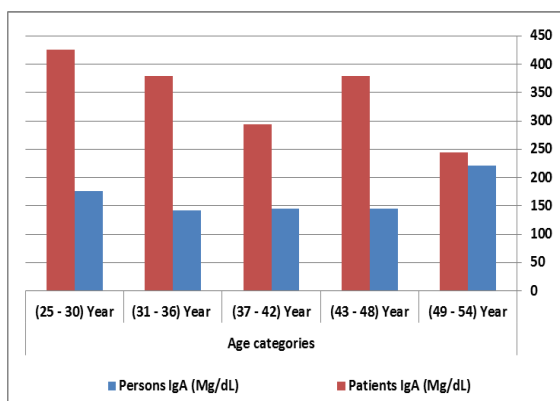
Comparison between of the antibodies types of the study groups in (Mg/dL)	Mean Difference	Stand. Error	Sig.
The IgA of the patients and apparently healthy persons	186.692	134.921	0.169
The IgM of the patients and apparently healthy persons	78.379	134.921	0.563
The IgG of the patients and apparently healthy	865.333*	134.921	0.001
The IgA of the male patients and male apparently healthy persons	173.501	223.925	0.442
The IgM of the male patients and male apparently healthy	17.726	223.925	0.937
The IgG of the male patients and male apparently healthy persons	649.501*	223.925	0.006
The IgA of the female patients and female apparently healthy persons	199.884	150.026	0.189
The IgM of the female patients and female apparently healthy persons	139.034	150.026	0.359
The IgG of the female patients and female apparently healthy persons	1081.167*	150.026	0.001
*The mean difference is significant at the 0.05 level			

Table (3) significant differences between all, males and females patients were 0.001, 0.006 and 0.001 respectively of the IgG when comparison with same groups of the apparently healthy persons. And no significant differences were presented of both IgA and IgM for all study groups.

**Table:4. Age categories and number of the study groups.**

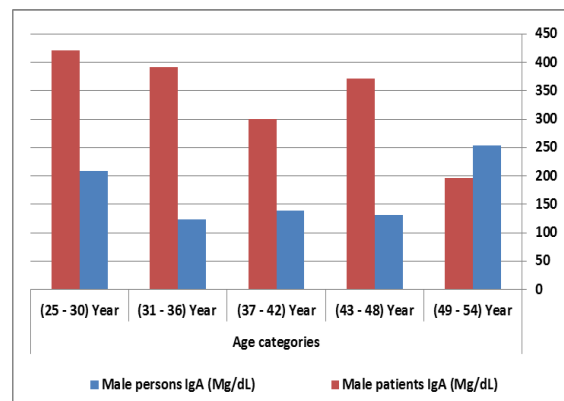
Age categories	Number of			Number of		
	Hepatitis -B- Patients	Male patients	Female patients	Apparently healthy persons	Male apparently healthy persons	Female apparently healthy persons
25 - 30	10	4	6	4	2	2
31 - 36	14	6	8	6	2	4
37 - 42	10	6	4	4	2	2
43 - 48	6	4	2	6	4	2
49 - 54	8	4	4	4	2	2
Total	48	24	24	24	12	12

Table (4) number of the patients and persons as well as number of the males and females within these groups, these placed in five categories of the ages, this used for drawn histograms between antibodies means for these age categories and comparison between these study groups as in figures numbered from (figure:2. to figure:10).



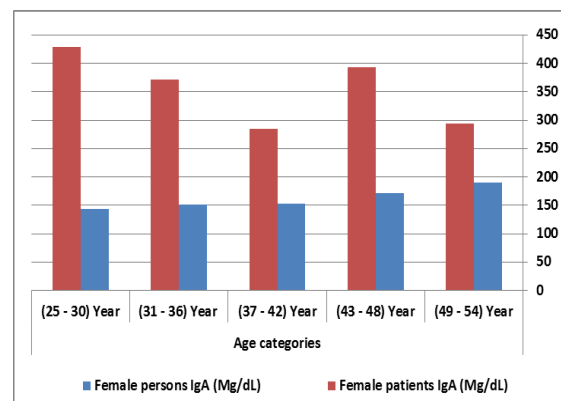
**Figure:2. Comparison between the concentration of the (IgA) in age categories of the patients and apparently healthy persons.**

Figure (2) elevation in the (IgA) antibody concentration of the patients in comparison with persons.



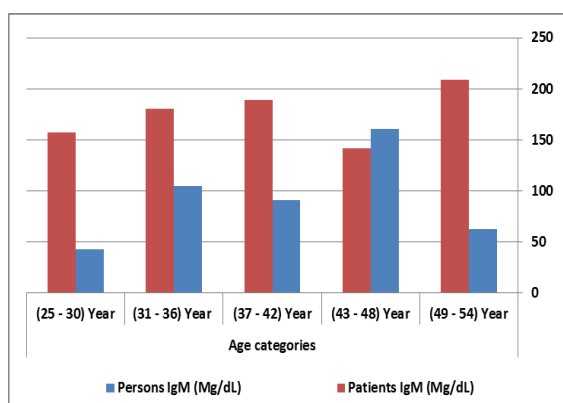
**Figure:3. Comparison between the concentration of the (IgA) in age categories of the male patients and male apparently healthy persons.**

Figure (3) raising in the (IgA) antibody concentration of the males patients in comparison with males persons except (49-54) age category.



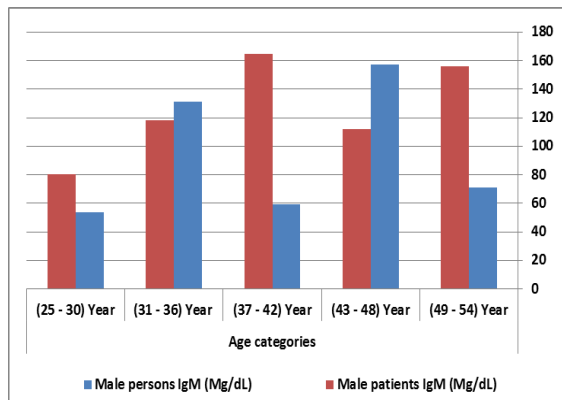
**Figure:4. Comparison between the concentration of the (IgA) in age categories of the female patients and female apparently healthy persons.**

Figure (4) increasing in (IgA) antibody concentration of the females patients in comparison with females persons.



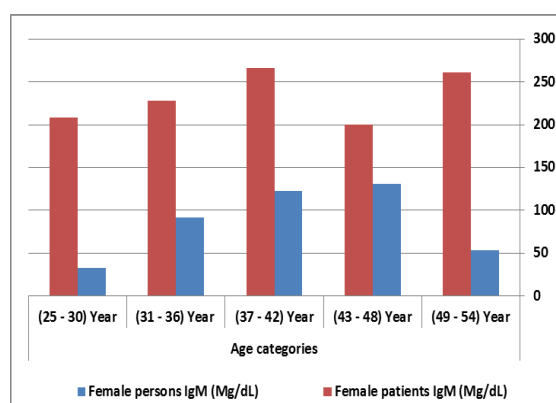
**Figurer:5.** Comparison between the concentration of the (IgM) in age categories of the patients and apparently healthy persons.

Figure (5) raising in the (IgM) antibody concentration of the patients in comparison with persons except (43-48) age category.



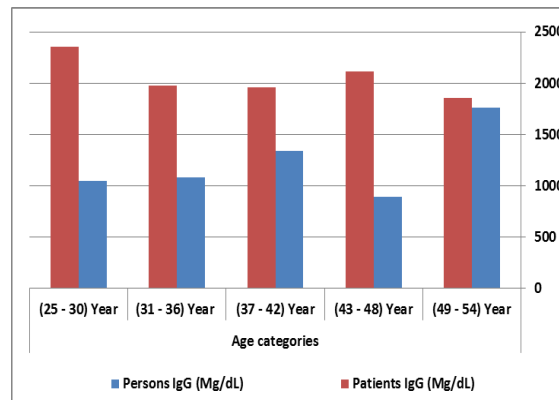
**Figurer:6.** Comparison between the concentration of the (IgM) in age categories of the male patients and male apparently healthy persons.

Figure (6) elevation in the (IgM) concentration of the males patients in comparison with males persons except (31-36) & (34-48) age categories.



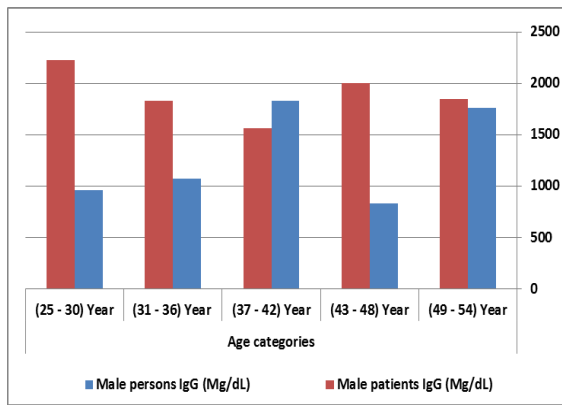
**Figurer:7.** Comparison between the concentration of the (IgM) in age categories of the female patients and female apparently healthy persons.

Figure (7) increasing in the (IgM) antibody concentration of the females patients in comparison with females persons.



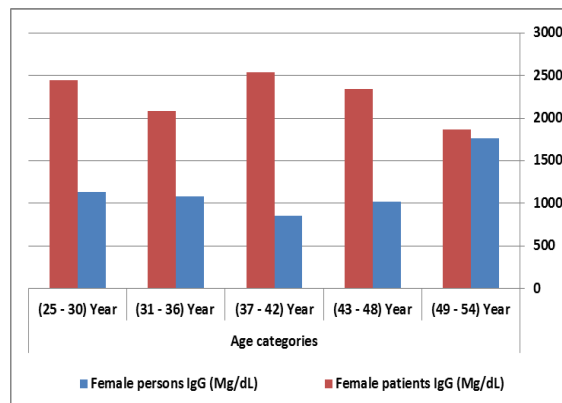
**Figurer:8.** Comparison between the concentration of the (IgG) in age categories of the patients and apparently healthy persons.

Figure (8) raising in the (IgG) antibody concentration of the patients in comparison with persons.



**Figuer:9. Comparison between the concentration of the (IgG) in age categories of the male patients and male apparently healthy persons.**

Figure (9) elevation in the (IgG) antibody concentration of the males patients in comparison with males persons except (37-42) age category.



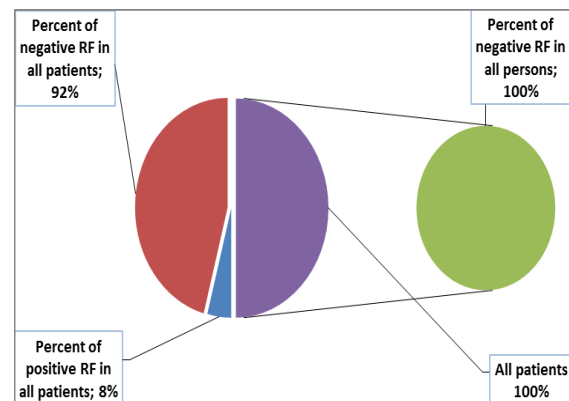
**Figuer:10. Comparison between concentration of the (IgG) in age categories of the female patients and female apparently healthy persons.**

Figure (10) increasing in the (IgG) antibody concentration of the females patients in comparison with females persons.

**Table:5. Percent of the Rheumatoid factor (RF) and C-Reactive protein (CRP) in the patients and persons.**

Result		Persons		Patients	
		RF	CRP	RF	CRP
All	Positive percent (%)	0 %	0 %	8.333 %	25 %
	Negative percent (%)	100 %	100 %	91.666 %	75 %
Male	Positive percent (%)	0 %	0 %	16.666 %	50 %
	Negative percent (%)	100 %	100 %	83.333 %	50 %
Female	Positive percent (%)	0 %	0 %	0 %	0 %
	Negative percent (%)	100 %	100 %	100 %	100 %

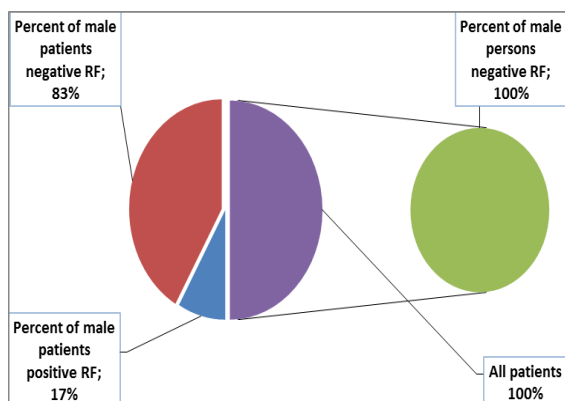
Table (5) all of the patients have positive percent of the (RF) and (CRP) were (8.333%) and (25%) respectively; while the males patients have positive percent were (16.666%) and (50%) for (RF) and (CRP) respectively; and the females patients have positive percent were (0%) for both (RF) and (CRP), whereas the apparently healthy persons have positive percent of both the (RF) and (CRP) were (0%).



**Figuer:11. Comparison between the percent of the Rheumatoid factor (RF) in patients and apparently healthy persons.**

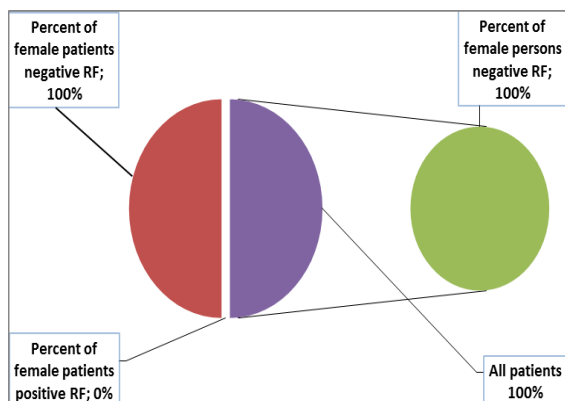
Figure (11) percent of the positive (RF) in patients was (8%), whereas the negative in patients and persons were (92%) and (100%) respectively.





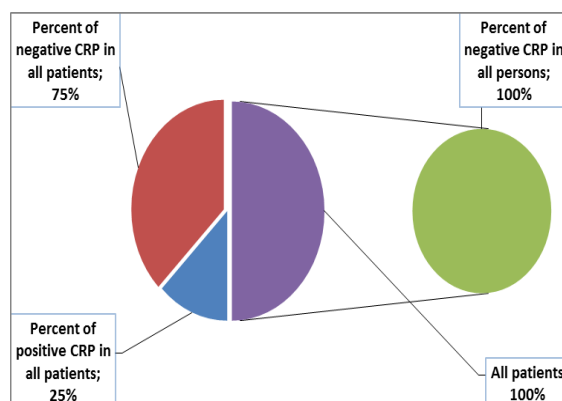
**Figurer:12.** Comparison between the percent of the Rheumatoid factor (RF) in male patients and male apparently healthy persons.

Figure (12) percent of the positive (RF) in males patients was (17%), while the negative in males patients and females persons were (83%) and (100%) respectively.



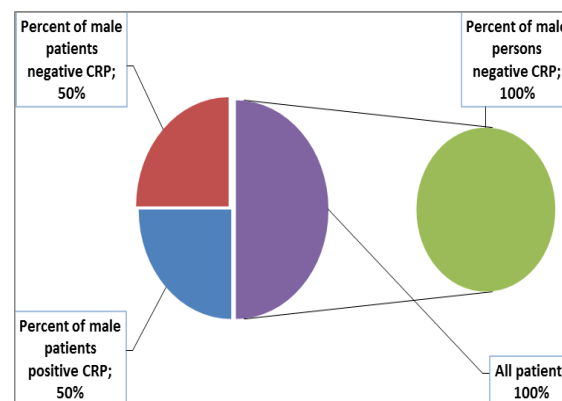
**Figurer:13.** Comparison between the percent of the Rheumatoid factor (RF) in female patients and female apparently healthy persons.

Figure (13) percent of the positive (RF) in females patients was (0%), whereas the negative in females patients and females persons were (100%) and (100%) respectively.



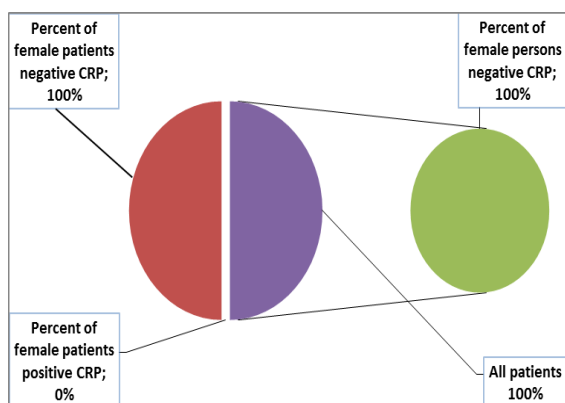
**Figurer:14.** Comparison between the percent of the C-Reactive Protein (CRP) in patients and apparently healthy persons.

Figure (14) percent of the positive (CRP) in patients was (25%), while the negative in patients and persons were (75%) and (100%) respectively.



**Figurer:15.** Comparison between the percent of the C-Reactive Protein (CRP) in male patients and male apparently healthy persons.

Figure (15) percent of the positive (CRP) in males patients was (50%), whereas the negative in males patients and males persons were (50%) and (100%) respectively.



**Figurer:16. Comparison between the percent of the C-Reactive Protein (CRP) in female patients and female apparently healthy persons.**

Figure (16) percent of the positive (CRP) in females patients was (0%), while the negative in females patients and females persons were (100%) and (100%) respectively.

### **Discussion:**

Table (2 and 3) explained the significant elevation in the concentration of the antibody (IgG) as well as no significant elevation in the concentrations of the both antibodies (IgM) and (IgA) of the patients when comparison the concentration of these antibodies with same antibodies of the apparently healthy persons table (1 and 3). And when comparison the present results with other workers, Stoica *et al* who found the IgM was remarkably higher while the IgG and IgA were significantly greater in patients than in controls [23]. Bersohn *et al* explained the IgM level was significantly higher while the levels of the IgA and IgG were not significantly different in the

hepatitis-B infected patients [24]. Joshi *et al* illustrated the All antibodies IgA, IgM and IgG were elevated significantly in HBV infections [25].

The responses of the adaptive immunity consist from the complexes of the network of the effector immune cell types, all of these plays a specific role in the elevation of the immunity against of the hepatitis B virus infections, the CD4 T-cell, named as the helper T-cell, are active for cytokines production and are important in the activation of the effector cytotoxic CD8 T-cell and antibody production B-cell type [26]. The CD8 T-cell pass on to clear of hepatitis B virus infected hepatocytes through the mechanisms of noncytolytic and cytolytic, also decreasing of the level of this virus [27].

The dendritic cells act as represent cells that production of the specialized antigens for necessary cells populations acting with elevation response of adaptive immune system [28]. With regard to their important role in T cell suppository, efficiently and working changes of the dendritic cells can explain the case of the lower responses within B-cell and T-cell in patients with chronic hepatitis B [26]. The B-cell can remove and prevent viral infections and/or reinfection through production of the antibodies [29].

Table (5) explained the positive percent of the rheumatoid factor (RF) in hepatitis B patients was (8.333%). As well as the results of the males and females patients and control were

illuminated in figures (11 to 13). And when comparison these results with other workers, Arai *et al* who found this factor was positive in (39.5%) of the all hepatitis B patients [30]. Dalkilic *et al* who found RF factor was positive in (18.7%) of the all HBV patients [31]. Choi *et al* who found RF factor was positive in (3.5%) of the all hepatitis B patients [16].

The patients that infected with hepatitis B virus explain elevation in the positivity rates of rheumatoid factor [32]. A chronic infections including hepatitis B produce high levels of this factor [33]. Recent studies demonstrated the HBV infections can increasing of specific B-cells activation [34]. In hepatitis B infections the HBeAg-antibody complex may play role in rheumatoid factor formation [32]. And production of this factor can by antigen specific B cells with help from T cells as a resulting of binding and processing of immune complexes in which IgG functions as antigen [35]. Another mechanism of RF production including cross reactivity between epitopes of antigen and/or auto antigen with antibody-G, as well as the polyclonal B cell activation during infections and responses can be another cause of rheumatoid factor formation [36].

Table (5) showed the positive percent of the C-reactive protein (CRP) in hepatitis B patients was (11.68%). As well as the results of the males and females patients and control were illustrated in figures (14 to 16). Ma *et al* who found this factor was positive in (3.5%) of the all Hepatitis B patients [37]. Liu who found this

factor was positive in (37.97%) of the all Hepatitis B patients with liver cirrhosis patients [38].

The protein of C-reactive is the reactant of acute phase associated with the tissue damage and liver inflammation [39]. This protein strongly associated with the chronic infections of the hepatitis B viruses and can reflect the severity of liver damage [40]. liver inflammations in patients infected with HBV have been confirmed to be mediated by the cytokines that may plays a specific roles in the pathogenesis of chronic infection with this virus [41]. This protein is manufactured in the acute phase of the inflammations in responses to interleukin-6 (IL-6) and an elevated in this protein suggests the presence of hepatic inflammation as a result of liver injury [42]. This protein is up regulated by cytokines such as interleukin-6 (IL6) and tumor necrosis factor (TNF) [43].

### **Conclusions:**

The IgG antibody elevation associated with hepatitis B virus infections as well as, increase in the concentrations of the Rheumatoid Factor and C-Reactive can be found in this disease. And these immune markers can be used for identification and explanation the progress of hepatitis B infections and diseases.

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