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Estimation of Interleukine -1 and Granulocyte Macrophage – Colony Stimulating Factors among Type a Hepatitis Patient and Autoimmune Hepatitis

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Abstract
Hepatitis A Virus (HAV) is the most common cause of acute hepatitis worldwide. Although infection with Hepatitis A virus is often mild and asymptomatic in young children, the disease can be severe in adults .The aimed of this study was estimate serum cytokines levels of interleukin (IL-1) and Granulocyte macrophage\colony stimulating factors (GM\CSF) with Hepatitis A virus(HAV) , autoimmune hepatitis (AIH) and apparently healthy individuals as a control group. Forty blood samples have been collected from HAV patients , 31samples of (AIH) patients , in addition to 24 apparently healthy individuals . The detection of (HAV),(IL-1) and (GM\CSF)were estimated by Enzyme Linked immuno sorbent assay (ELISA) method. The present study shows that the high frequency of disease in age 20-29 years most of them male more than female. The mean of the elevated IL-1 in serum of HAV, AIH and healthy control was 59.44 ± 27.23, 26.39 ± 13.35 and 4.97 ±2.78 respectively, while the mean of elevated GM-CSF in serum of the studied groups was 48.66±25.41, 12.4±3.74 and 1.71±0.88 respectively. IL-1 appears in 39 (97.5%) of HAV patients which was elevated in comparison with 27 (87.1%) AIH group. Level IL-1 among AIH was observed highly significantly elevated in comparison with healthy control ($P<0.01$). The elevated of GM-CSF in this study was showed that 40(100%) patients with HAV were appeared of abnormal GM-CSF, while 31(100%) AIH patients were observed for abnormal GM-CSF in AIH compared with healthy control.. The results indicating that highly significant relationship for the studied group ($P<0.01$). This study concluded that Sreum IL-1 and GM-CSF was elevated in both HAV and AIH as mediating acute inflammatory reactions and chemokine respectively compared with healthy controls.
Keywords: Interleukine -1, Granulocyte Macrophage –Colony Stimulating Factors ,type A Hepatitis autoimmune hepatitis

Introduction

Viruses with a positive-strand RNA genome include the viral families Picornaviridae, Togaviridae, Flaviviridae, Caliciviridae, and Coronaviridae. The viruses in these families cause a broad spectrum of diseases, but share some features such as they replicate in the cytoplasm so genomic RNAs serve as messenger RNAs and are infectious . Genomic RNAs are non-segmented , virions do not contain any enzymes and virus-specified proteins are synthesized as poly proteins that processed by viral and cellular proteases, giving rise to individual viral proteins (Harvey *etal*. 2007) **.** Hepatitis A is an infection of the liver caused by hepatitis A virus (HAV), is a nonenveloped, spherical, positive stranded RNA virus, classified within the genus hepatovirus of the picornavirus family, usually transmitted by the fecal-oral route through interpersonal contact . The disease is generally mild and does not lead to chronic or persistent hepatitis., but severity tends to increase with age. Asymptomatic disease is common in children. Jaundice may occur in 70–80% of those infected as adults. Fulminant hepatitis can occur but is rare. There is no chronic carrier state and chronic liver damage does not occur (Bell *et al*. 2001) . HAV has a worldwide distribution . The prevalence varies from one population to another and it related to socioeconomic factors and living standard of the population (Dienstag *etal*. 1978, . Gust *etal*.1988) . Most hepatitis A outbreaks are due to fecal-oral route of transmission because of contamination of food or water with sewage. In developing countries with poor living conditions such as inadequate water supply, poor sewage facilities and sanitary conditions, the level of transmission of HAV within the community is high .Improvement in hygienic and socio-economic conditions has resulted in a decrease in the number of natural childhood infections (Hadler 1991) **.**So there are multiple mechanisms to control innate and adaptive immune response to activated of hepatitis A viral infections in the host, during infection virus specific cytotoxic T lymphocyte and helper CD4 T cells play key effectors and regulators role in immune responses against hepatotropic virus (Fierr *etal*. 2013) .So IL-1 is one of cytokines play key roles in mediating acute inflammatory reactions while Granulocyte-Macrophage Colony Stimulating Factor(GM-CSF) act as chemokine (Shaikh 2011) , that perpetuate eosinophil activation and survival the source of GM-CSF may be the alveolar macrophages which are reported to produce two to threefold higher levels of GM-CSF than control macrophages.a (Paul & Ruddle 1988) .to improve the immunological function of patients with various

diseases and to ameliorate hematological disorder (Bahagat *etal.*2011).

Materials and methods

A total of 40 hepatitis A Iraqi patient, (32male to 8 female) 31 autoimmune hepatitis patients(23 female to 8 male) as disease control group and 24 Apparently healthy individuals (10 female to 14 male) were enrolled in this study. These patients were attending the medical city hospital/ Baghdad during the period between July/2013 to August/ 2013. Each patient suffering from fatigue, fever, nausea, loss of appetite and jaundice. Diagnostic by gastrointestinal tract`s(GIT) physician . The control group included individuals was apparently healthy who matched patients in sex and age.

Sample collection : Five ml of blood was collected from each subject and then serum was separeated and distributed into three eppendorff tubes for ELISA methods and then kept it in freez at -20 °C .the diagnosis of 40 individuals hepatitis A was confirmed by serologic tests (IgM and total anti-HAV antibodies) were detected by enzyme-linked immunosorbent assay ELISA (Biokit , Spin). All studied groups were examined for qualitative diagnostic test to detection IL-1by ELISA according to the manufacturer's protocol (Human , Germany) . One hundred µL of standard, serum samples and serum control per well was added to antibody coated wells and incubated for 1 h at room temperature , the wells was washed by washing buffer (1:25), then100 µL of IL-1 conjugate also was added. Plate was covered with cover plate and incubated for two hours at room temperature, with shaking , the wells was washed again . Addition of 200µL of TMP substrate was added to each wells and then incubated for 30 minutes at room temperature in the dark, with shaking. The reaction was terminated by adding Fifty µL of stop solution into the wells. The absorbance was measured at 405 nm. Immunological assay for Estimation of serum GMC/SF level (Human , Germany) for all studied group By ELISA test intended for the qualitative detection dependent of the manufacturer's protocol . To antibody coated wells 100 µL of standard, samples and control was added for each well and incubated for 1 h at room temperature, the wells was washed by washing buffer (1:25), then100 µL of GM\CSF-1cojugate also was added. Then the plate was covered and incubated for two h .at room temperature with shaking , the wells was washed again . Two hundred µL of TMP substrate was added to each wells and then incubated for 30 min. at room temp. in the dark, with shaking. The reaction was terminated by adding Fifty µL of stop solution into the wells. The absorbance was measured at 405 nm. Statistical analysis : Data was put on compute file for storage and analysis . SPSS version 18 (statistical package for social science) was used for data analysis include Mean value, Standard Deviation, Standard Error, and (95%) Confidence interval for population , Odds Ratio for (Normal**:** Abnormal) responding between the two independent groups , Contingency coefficient for the association tables and Inferential data analysis (The One-Sample Kolmogorov-SmirnovTest , analysis of Variance (ANOVA) P value ≤ 0.05 was considered statistically significant , analysis for Homogeneity of variances) .

Result and Discussion

The results in table 1 was showed that high frequency of studied group in age 20-29 years with (12,10,10) at high percentage (30, 32, 41,7%) for hepatitis A and autoimmune, healthy control respectively.

Table 1: Distribution of The Studied Samples According to Age Groups

Age Groups (Years)	Groups			C.S. (*) P-value
	Type-A	AIH**	Healthy	
10 - 19	11	5	1	C.C.=0.370 P=0.131 NS
	27.5%	16.1%	4.2%	
20 - 29	12	10	10	
	30.0%	32.3%	41.7%	
30 - 39	7	6	8	
	17.5%	19.4%	33.30%	
40 - 49	5	8	4	
	12.5%	25.8%	16.7%	
50 - 59	1	2	1	
	2.5%	6.5%	4.20%	
60 - 69	4	0	0	
	10.0%	0.0%	0.0%	

(*) **NS= Non Significant at P> 0.05, (**) AIH=Autoimmune hepatitis**

The result of the current study demonstrated that the highest frequency for the disease (30%) was among patients at 20-29 years of age. In comparison with that of Kanra *etal*. (2002) in Turkey who found that 90.9% of patients were at 20-24 years and 91.1% at age 25-29 years. On the contrary, Morris *et al*.,(2002) manifested that 17% of patients at 20-29 years, though it seems to be that there was sharp increment in

seroprevalence above 30 years (73.5%) in England, so as in Australia. Amin *et al.,* (2001) found 50% of patients at 40 years, while the rate of infection in older people was 61%. In Palestine it was demonstrated that 87.8% at 6 years had already acquired the infection, the prevalence increase gradually but linearly with age 14 years at 97.5% (Tanır *etal*. 2003). On the other hand, adult AIH often presents actually and has a more aggressive course that in middle-age and elderly patients(Mieli-Vergain & Vergani 2009) .Also this difference supports that disease incidence varies geographically with wide differences in prevalence from country to country, even within same country or city, the incidence may also vary within time (Koff 1998 . Tanır *etal.* 2003) .

The results in Table 2 shows that the mean ± SD of IL-1 concentrations among the sera of HAV, AIH and healthy control were (59.44±27.23, 26.39±13.35 and 4.97±2.78 µg/ mL respectively).

Table 2: Statistical difference in serum IL-1 of the studied groups

The Groups	No.	Mean	Std. Dev.	Min.	Max.
Type – A	40	59.44	27.23	8.0	102
Auto-Immune Hepatitis	31	26.39	13.35	5.8	60.7
Healthy control	24	4.970	2.780	1.0	11.2

Table 3 shows the results of ANOVA technique for testing equality of mean values of "IL-1" readings among different of the studied groups. The results show that a highly significant different are accounted among the studied groups at P value P<0.01. The results of the present study reveals that the serum levels of IL-1 were significantly elevated in all patients with HAV in comparison with AIH and healthy control

Table 3: Means and equality of variances for IL-1 parameter at the studied groups

Parameters	ANOVA for Equality of Means		C.S. (*)
	F - statistics	Sig. (*) (2-tailed)	
Interleukin-1	63.859	0.000	HS

(*) **HS: Highly Significant at P< 0.01**

For exploring the nature of real /or actual differences among studied groups, multiple comparisons through applying a suitable method should be applied to illustrated the probability levels of rejects the statistical hypothesis, and with respect to that we needs to be continuing testing for comparisons by using (Games-Howell) method. This test is appropriate when the variances are unequal assumed and that illustrated in table (4).

Table 4: Multiple Comparison (Games-Howell) of IL-1between pairs of parameter According to different samples

Group	Group	Mean	Sig. (*)	C.S. (*)
Type – A with	Auto-Immune Hepatitis	33.045	0.000	HS
Type – A and	Healthy	54.464	0.000	HS
Auto-Immune Hepatitis with and	Healthy	21.419	0.000	HS

(*) **HS: Highly Significant at P< 0.01**

Regarding to the subjects of "IL-1" parameter, the results were showed a highly significant different at P<0.01 among all pairs of comparisons .The current study shows that there was statistically significant difference between the means of Type A with AIH was observed fewer than Type A with healthy; due to

secreted IL-1 from AIH fewer than HAV but more than healthy. Finding indicates that IL-1 secretion with HAV more that AIH because the IL-1 is a master cytokine in inflammation and it regulates diverse cellular process during both acute and chronic infections. It induces potent inflammatory molecules such as COX2, nitric acid and TNF-α (Dinarello 1984 , Samad *etal.* 2001) and the mean of Type A with healthy were significant differences due to there are no foreign pathogen that induction for production of IL-1 and GM-CSF in healthy control.

Table 5 shows that the mean ±SD of concentration for GM-CSF to HAV, AIH and healthy control were 48.66±25.41, 12.4±3.74 and 1.71±0.88 respectively.

Table 5: Distribution of GM-CSF at the studied groups

Groups	No.	Mean	Std. Dev.	Min.	Max.
Type - A	40	48.66	25.41	11	95.7
Auto-Immune Hepatitis	31	12.40	3.74	5.8	20
Healthy	24	1.71	0.88	0.6	3.6

The current study findings show a markable increase of GM-CSF due to IL-1 contributes to myeloid cell differentiation by increasing the expression of growth factors such as G-CSF and GM-CSF(- Dinarello 1984) . Table 6 shows the results of ANOVA technique for testing equality of mean values of GM- CSF readings among different of the studied groups. The results show that a highly significant different are found among the studied groups at P value P<0.01 . The results of this study reveals that the serum levels of IL-1 were significantly elevated in all patients with HAV in comparison with AIH and healthy control

Table 6 : Means and equality of variances for GM-CSF parameter at the studied groups

Parameters	ANOVA for Equality of Means		C.S. (*)
	F - statistics	Sig. (*) (2-tailed)	
GM-CSF	72.397	0.000	HS

(*) **HS: Highly Significant at P< 0.01**

The present study shows that there were statistically high significant differences between the mean of type A with AIH at 36.258 fewer than type A with healthy control 46.949 (P<0.01) due to the secretion of GM-CSF from type A more than AIH because the correlation between IL-1 secretion and GM-CSF. On the other hand, the mean of AIH compared with healthy control was 10.962 (P=0.01) due to the elevated of GM-CSF secretion from AIH compared with healthy control .Result are shown in table 7 .

Table 7: Multiple Comparison (Games-Howell) of GM-CSF among all pairs of parameter According to different samples

Group	Groups	Mean	Sig. (*)	C.S. (*)
Type – A with/ and	Auto-Immune Hepatitis	36.258	0.000	HS
	Healthy	46.949	0.000	HS
Auto-Immune Hepatitis	Healthy	10.692	0.000	HS

(*) **HS: Highly Significant at P< 0.01**

Regarding to the subjects of "GM-CSF" parameter, the results showed a highly significant different at P<0.01 among all pairs of comparisons. According to the previous suggested technique, Figures (1) and (2) represent the cut off point for abnormal (GM-CSF) parameters readings respectively. Therefore, the cut off point for IL-1was 11.2 while in GM\CSF was 3.6.

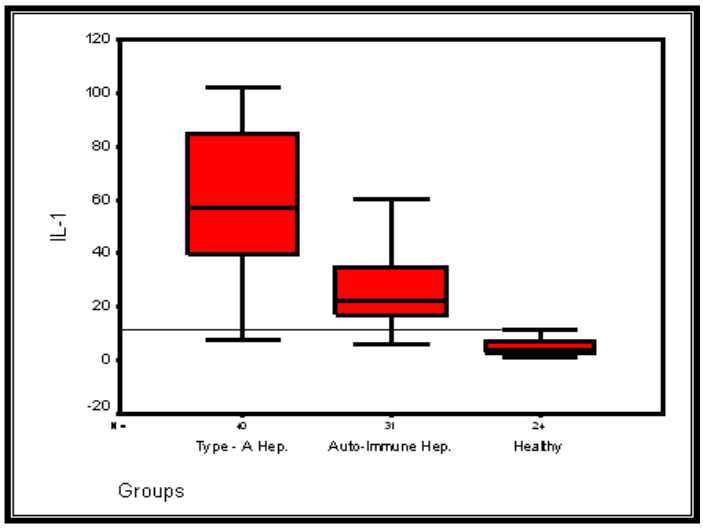

Figure 1: Stem-Leaf Plot for the studied parameter (IL-1) to estimate cutoff point for the studied groups

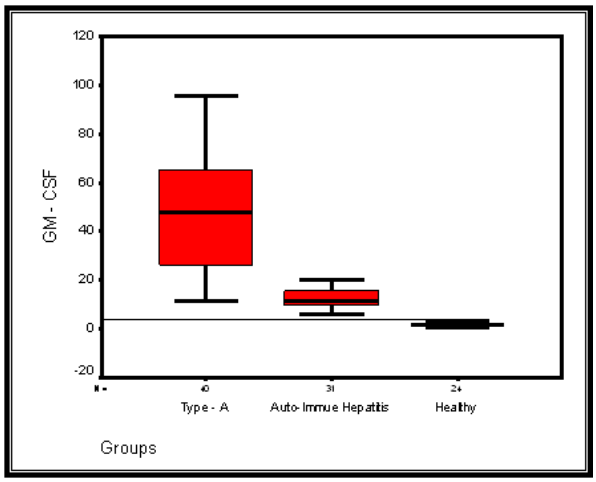

Figure 2: Stem-Leaf Plot for the studied parameter (GM-CSF) to estimate cutoff point for the studied groups

Table 8 represents the redistribution of normal and abnormal individuals for (IL-1) at the studied groups (Auto-Immune Hepatitis & Control) according to all probable combination with cause correlation ships due to the (cutoff point), The results shows that 39(97.5%) patients were observed of abnormal IL-1 in HAV and 27 (87.1%)of AIH compared with healthy control. There was statistically highly significant differences between both (P=0.0001). The current study findings shows remarkable increase of IL-1 secretion. In contrast of Muller *et al.*,(1989) who found a reduced IL-1 production by peripheral blood monocytes in acute viral hepatitis due to the acute phase response in only moderate during the disease or for blunted acute phase response could be reduction in hepatic IL-1 receptors (Dower . & Urdal 1987) during acute viral hepatitis or direct alteration of protein synthesis in infected hepatocytes (Muller *et al.*, 1989). The increase of IL-1 (α and β) and TNF are extremely potent inflammatory molecules, they are the primary cytokines that mediated acute inflammation induced in animals by intradermal injection of bacterial lipopolysaccharides and two of the primary mediators of septic shock (Feghali & Wright 1997) .Also interleukins play a major role in the development of the inflammatory process, fibrosis and the regeneration of the liver. A very important role in the escalation of the inflammatory cytokines including IL-1, IL-4 and IL-6 (Missal *etal.*1995 , El-Ghaffar *etal.* 2008) .

The result shows that a meaningful correlation had been reported with (C.C. =0.695) and that indicating a highly significant relationship at P<0.01 for the type A and control groups, as well as a for the Auto-Immune Hepatitis and control groups.

Table 8: Distribution of normal and abnormal status between the studied parameter (IL-1) in the studied samples and healthy group with cause's correlation ships

Groups	Freq. & Percentage	Status	
		Normal	Abnormal
Type – A Hepatitis	Freq.	1	39
	% Groups	2.5%	97.5%
	% IL-1	4.2%	100%
Healthy	Freq.	24	0
	% Groups	100%	0.0%
	% IL-1	96%	0.0%
CORRELATION Coefficient P-value C.S. (*)		C.C.=0.695 P=0.000 HS	
Auto-Immune Hepatitis	Freq.	4	27
	% Groups	12.9%	87.1%
	% IL-1	14.8%	100%
Healthy	Freq.	24	0
	% Groups	100%	0.0%
	% IL-1	85.7%	0.0%
CORRELATION Coefficient P-value C.S. (*)		C.C.=0.654 P=0.000 HS	

(*) **HS: Highly Significant at P< 0.01**

Table 9 represents the redistribution of normal and abnormal individuals for (GM-CSF) in the studied groups (Type A Hepatitis, Auto-Immune Hepatitis & Control) with cause relationships according to the (cutoff point). The result showed that 40 (100%) patients with HAV were appeared of abnormal GM-CSF, while 31(100%) patients were observed for abnormal GM-CSF in AIH compared with healthy control. The highly significant differences observed between both at P<0.0001. The increase of IL-1 and GM-CSF is due to these cytokines play key roles in mediating acute inflammatory reaction (Feghali & Wright 1997) .These factors are not simply proliferative stimuli but they can also regulate the function, activity of mature cells(Metcalf 1986) .These factors widely produced in the body as regulators, probably play an important role in resistance to infections (Metcalf 1985)..The result shows that a meaningful correlation had been reported with (C.C. =0.7.7) and that indicating a highly significant relationship at P<0.01 for the type-A and control groups, as well as for the Auto-Immune Hepatitis and control groups.

Table 9: Distribution of normal and abnormal of the studied parameter (GM-CSF) in the studied samples with cause's correlation ships

Status	Freq. & Percentage	Groups	
		Normal	Abnormal
Type – A Hepatitis	Freq.	0	40
	% GM-CSF	0.0%	100%
	% Groups	0.0%	100%
Healthy	Freq.	24	0
	% GM-CSF	100%	0.0%
	% Groups	100%	0.0%
Correlation Coefficient P-value C.S. (*)		C.C.=0.707 P=0.000 HS	
Auto-Immune Hepatitis	Freq.	0	31
	% GM-CSF	0.0%	100%
	% Groups	0.0%	100%
Healthy	Freq.	24	0
	% GM-CSF	100%	0.0%
	% Groups	100%	0.0%
Correlation Coefficient P-value C.S. (*)		C.C.=0.707 P=0.000 HS	

(*) **HS: Highly Significant at P< 0.01**

Conclusion

Sreum IL-1 and GM-CSF was elevated in both HAV and AIH as mediating acute inflammatory reactions and chemokine respectively compared with healthy controls.

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