Original Article

Serum interleukin 1 and interleukin 10 levels in Iraqi leukemic patients with hepatitis G virus infection.

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Summary:

Background: Hepatitis G virus and GB virus C (GBV-C) RNA viruses that were independently identified in 1995, and were subsequently found to be two isolates of the same virus. Blood transfusion is the main risk factor for HGV transmission Immune-mediated mechanisms are believed to play an important pathogenic role in hepatitis G virus infection. Interleukin-1 (IL-1) plays an important role in the inflammatory process. implying that IL-1 may play a role in viral clearance and suggesting that IL-1 has direct antiviral activity, so that IL-1 induces novel antiviral pathways within a cell. Interleukin 10 (IL-10)is secreted by T helper-2 type cells (Th2) which may down regulate cell-mediated immune effecter mechanisms important in the host defense against intracellular pathogens.

Objectives : This prospective study aimed to estimate interleukins 1 and 10 levels in acute hepatitis (G) in Iraqi patients with leukemia

Methods: This cross sectional study was carried out at the national center of hematology , almustansiriya University and department of microbiology in Baghdad medical college in Baghdad, Iraq from January till August 2012. The study involved 33 patients (19 males & 14 females) diagnosed with hepatitis G infection as having a positive IgM antibodies by ELISA technique .Serum IL-1 and IL-10 levels were measured in 33 patients with hepatitis G virus infection and 40 leukemia patients without HGV infections as a control group by ELISA technique .

Results: Twenty three patients (70%) with hepatitis G infection had elevated serum IL-1 alpha levels (the normal detection level is below 5 pg/ml), the patient serum IL 1 alpha range 2-37 pg/ml, with a mean 27.813 and SD 32.765). There was also a high significant correlation between serum IL-1 alpha level and hepatitis G infection (p value= ± 0.004) when compared with the control group by the t –test Also serum IL-10 level was below the normal detection limit (5-20 pg/ml) in 20 (60%) patients .The 33 patients had serum IL-10 levels (range 0.6 - 9 pg/ml, with a mean 2.213 & SD± 4.745) . There was a significant correlation between serum IL-10 level & hepatitis G infection when compared with the control group ($p<0.0386$ by t- test),so the viral infection may suppress the T helper 2 cells by the gamma interferon . This elevation in IL1 alpha which is an inflammatory cytokine produced by antigen presenting cells , may suggest that IL-1 is an important interleukin to eradicate hepatitis G virus infection.

Conclusions: This study showed that serum Th2 cytokines are suppressed (IL10) while serum inflammatory cytokine (IL 1 alpha) is elevated in a proportion of patients with hepatitis G virus infection. However, the elevated IL 1 alpha cytokine levels may represent a systemic response and not as a result of increased local production within the liver.

Keyword : Hepatitis G virus , interleukin 1, interleukin 10.

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Introduction:

Two different laboratories in the USA isolated a new flavivirus- like RNA virus in the years 1995 and 1996. The first laboratory named it “G B virus-C (GBV-C)” and the other “hepatitis G virus (HGV)”. Both viruses were subsequently considered different genotypes of the same virus because they were found to share most of the nucleotide and amino acid sequences (1). Both viruses have a single stranded RNA genome of approximately 9.4 kb (2). They are member of the Flaviviridae family and are phylogenetically related to hepatitis C virus, but appear to replicate primarily in lymphocytes, and poorly if at all in hepatocytes (3). (4). Parenteral, sexual and vertical transmission of (HGV) have all been documented, and because of shared modes of transmission, individuals infected with HIV are commonly co-infected with (HGV) (5)(6). Although the precise transmission rate is unknown, an elevated prevalence of HGV-RNA has been described in subjects at risk for parenteral infections, such as blood and blood products recipients, intravenous drug abusers, and patients on hemodialysis(6-8). Some studies reported that HGV prevalence in healthy blood donors ranged from 1.2 to 4.2 percent(9-11). Interleukins are soluble mediators of the immune system synthesized and secreted by leukocytes in extremely low concentration (picomolar and nanomolar)and manifest their biological effect

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through specific receptors expressed at the surface of their target cells, the central role of their action is cell to cell communication (12)Interleukin-1 (IL-1) plays an important role in the inflammatory process, IL-1 production can effectively inhibit HGV subgenomic RNA replication and viral protein expression, suggesting that IL-1 has a direct antiviral activity. In addition, IL-1 can induce one of the interferon-stimulated genes (ISGs), 1-8U, which exhibits antiviral activity., suggesting that IL-1 induces novel antiviral pathways(13) . Interleukin IL-10 is a potent anti-inflammatory cytokine derived from macrophages and T helper-2 type cells (Th2) which may down regulate cell-mediated immune effecter mechanisms important in the host defense against intracellular pathogens (14).

Patient and Methods:

This cross sectional study was carried out at the national center of hematology , almustansiriya University and department of microbiology in Baghdad medical college in Baghdad, Iraq from January till August 2012. The study involved 33 patients (19 males & 14 females) diagnosed with hematological malignancies , and 40 leukemia patients without HGV infections as a control group. Written informed concents were obtained from all patients and controls . the study was approved by the ethical committee of the microbiology department in Baghdad university and national center of hematology in Almustansiriya university. The age and gender ratios were similar in the groups of patients and controls: median age 34.3 years (range 16-60) and 33.4 years (18-55), M/F is 1.35:1 and 1.5:1 respectively. All patients and controls were HIV negative and the control had never been transfused; no other risk factors for blood-borne diseases were present in the controls, . All patients underwent blood tests and lymph node and bone marrow biopsy in order to diagnose and characterize the hematological malignancy.Five milliliters of blood samples were withdrawn from patients and controls. They were centrifuged and serum was separated. Serum was stored in aliquots at -20°C. Repeated freezing and thawing was avoided. All the biochemical parameters were done by routine laboratory methods unless otherwise stated. HGV IgM was detected with commercially available enzyme linked immunosorbent assay (CUSABIO BIOTECH CO.. LTD), while IL1 & IL10 by USBiological Bioassay ELISA kit USA biological Swanmpscott, Massachusetts 01907 www.usbio.net for research use only. The results were expressed as mean ± SD of mean usung statistical package for Social Sciences SPSS version 19.0 & Microsoft excel 2010 for data processing . Statistical significant difference was if $P<0.05$ and highly significant difference was $p<0.001$.

Results:

There were no significant differences in age, gender between the two group ($p> 0.05$) table(1) , twenty three patients (70%) of the thirty three patients with hematological malignancies and acute HGV infection had a high serum IL 1 level when compared to the control group and the mean value of serum IL1 in those patients had a highly significant elevation when compared with the control group with a P value < 0.004 as in table (2), while the result that for IL10 there was a significant depletion in the mean serum level of the patients as compared to the controls with a P value < 0.038 as shown in table (3) , and 20 (60%) of the patients had lower serum IL 10 below the normal detection limit which is (5)pg/ml.

Table 1.The demographic and clinical characteristics of all patients in study group

Characteristic	Patients	Controls	P value
Number of patients	44	40	
Age - Median (years) Range(year)	34.3 16-60	33.4 18-55	NS
Sex (male/Female)	19/14	24/16	NS
Primary Diseases	Patient Number (%)	Control Number (%)	
AML	8(24.3%)	9(22.5%)	NS
ALL	9(27.3%)	11(27.5%)	
CML	11(33.3%)	13(33.3%)	
CLL	5(15.1%)	7(16.7%)	

NS= not significant, AML; Acute myeloblastic leukemia, ALL; Acute lymphoblastic leukemia,CML; Chronic myelocytic leukemia, CLL; Chronic lymphocytic leukemia

Table (2): The difference in mean serum interleukin 1alpha level (pg/ml) between hepatitis G virus infected patients and the healthy control group

Study group N =73			
Values	Patient Group	Control Group	P value
Number	33	40	
Mean IL 1level (pg/ml)	27.813	11.929	0.004
SD (pg/ml)	32.765	7.764	
P value	Highly significant		

Table (3):The difference in mean serum interleukin (10) level (pg/ml) between hepatitis G viru infected patients and the healthy control group

Study group N =73			
Values	**Patient Group**	**Control Group**	**P value**
Number	**33**	**40**	
Mean IL 1level (pg/ml)	**2.213**	**7.129**	**0.0386**
SD (pg/ml)	**4.745**	**12.666**	
P value	**Significant**		

Discussion:

HGV or the GB virus C (GBV-C) have recently been isolated in patients with acute, chronic or post-transfusion hepatitis (12,13). It is now absolutely clear that HGV and its variant, GBV-C, are prevalent agents with a high carrier rate in the volunteer donor population(8,13). Although there has been a rapid increase in the epidemiologic and molecular knowledge of this virus, its clinical relevance remains largely unresolved(6)(15). Incidence of HGV between patient with hematological malignancies is higher than normal population as multiple blood transfusions are the most important risk factor for viral infections, despite of strict screening programs for HBV and HCV in blood bank, HGV should be put into consideration in screening program to prevent and decrease this serious infection (3). Our findings clearly showed that patients with hematological malignancies are frequently subjected to HGV due to multiple blood transfusions and that they suffer from severe immunosuppression, which has a significant influence on the clearance of HGV viremia and the production of anti-E2 immune response(16). Interleukins are soluble mediators of the immune system , the central role of their action is cell to cell communication (12)Interleukin-1 (IL-1) had a statistically significant elevation in HGV infected patients as compared to the control group so IL-1 may play an important role in the inflammatory process that occurs in HGV infection to inhibit HGV RNA replication and viral protein expression, suggesting that IL-1 may has a direct antiviral activity , or IL-1 can stimulate interferon-production , which exhibits antiviral activity., suggesting that IL-1 induces novel antiviral pathways(13) . Interleukin IL-10 is a potent anti-inflammatory cytokine derived from macrophages and T helper-2 type cells (Th2) which may down regulate cell-mediated immune effecter mechanisms that is important in the host defense against intracellular pathogens , so IL 10 suppresses cellular immunity , and this may lead to severe viral infection or even chronicity of the disease (14). So IL10 level is low in hepatitis infection ,while those with high serum level of IL10 may result in suppression of cellular immunity that may lead to chronic hepatitis infection (17). In conclusion , this study showed that there is significant increase in the serum level of IL 1 and low serum level of IL10 in patients with acute hepatitis G infection. . Despite of strict screening programs for HBV and HCV in blood bank, HGV should be put into consideration in screening program to prevent and decrease this serious infection.

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