Study Some of Natural killer Cell Phenotypes in Viral Hepatitis

Azza M. EzzEldin[1], Shereen P. Aziz[2], Ashraf K. Mohamed[2], Ali A. Ghweil[3], Mohamad M. Helal[3] , Esra Ahmad[2]

[1]Department of Clinical Pathology, Assuit Faculty of Medicine, Assuit University
[2]Department of Clinical Pathology, Sohag Faculty of Medicine, Sohag University
[3]Department of Tropical Medicine & Gastroenterology.Qena Faculty of Medicine, South Valley University

Abstract

Background& study aim: Natural killer (NK) cells are an integral part of the innate immune system. They have been suggested to play an important role in both defense against viral hepatitis and the pathogenesis of other liver diseases. Our study aims to investigating the expression of natural killer cells in viral hepatitis patients and if there is difference in those patients when compared with normal population

Patients & Methods: NK cell markers (CD3, CD56, and CD16) were studied by flowcytometery from 96 individuals including 76 patients with hepatitis B and C as well as 20 healthy subjects as control.

Results: Infection with viral hepatitis was associated with increased frequencies of $CD3^-$&$CD56^+$NK cells and $CD3^-$ &$CD16^+$ NK cells in the peripheral blood; and severity of hepatitis is correlated with the percentage of NK cells in peripheral blood with no statistically significant difference between HCV and HBV infections.

Conclusion: NK cell phenotypic changes can equally be observed in HBV and HCV infections and percentage of NK cell differ with severity of hepatitis. So this study suggests that the alteration of NK cell phenotypes with viral hepatitis depend on disease activity rather than virus-specific factors.

Keywords: NK cells; HBV; HCV; viral hepatitis.

Introduction

Human immunity is classically divided into innate and adaptive components. The adaptive immune response is generally regarded as being uniquely mediated by B and T lymphocytes. NK cells are now considered part of the family of innate lymphoid cells (ILCs), a classification of innate immune cells that mirrors that of CD8 and CD4 T cells in the adaptive immune system. NK cells represent the cytotoxic arm of the ILC family **[31].**

NK ($CD3^-$&$CD56^+$) cells mediate protective innate immune responses against viral infections. Their frequencies may decline in chronic HCV disease**[15,23]**, though other investigators challenged these results**[17,22,30]**. This may reflect variations in the methods used to identify NK cells. NK cells with different functions can be distinguished by their expression of CD56 **[9].**

NK cells are the major lymphocytic population in the liver, they are activated during acute HCV infection and remain activated during chronic HCV with an aberrant phenotype**[2]**.Several studies have reported reduced frequencies of NK ($CD56^+CD3^-$) cells in chronic viral infections including HBV, HCV and HIV although these findings are controversial **[6,25].**

$CD3^-CD56^{+dim}$ NK cells, which are cytotoxic against virally infected cells, may be important in hepatitis C virus (HCV)-infected patients who are successfully treated with pegylated interferon (PEG-IFN)-α. $CD56^+$ NK cells, which comprise approximately 10% of human peripheral blood mononuclear cells (PBMCs) and up to 30% of intrahepatic mononuclear cells **[24]**.

Most NK ($CD56^+CD3^-$) cells express the FcγIII receptor (CD16) which mediates antibody-dependent cell cytotoxicity**[8]**.They are critical for innate antiviral host defense since these cells have the capacity to limit viremia before adaptive T and B cell antigen-specific responses emerge**[5].**

NK cells can be divided into two subsets, dim and bright, according to CD56 surface density expression**[13].** Dim ($CD3^-CD56^{+dim}$ $CD16^+$) NK cells are cytolytic and comprise more than 90% of $CD3^-$ $CD56^+$ NK cells, whereas bright ($CD3^-CD56^{+bright}$ $CD16^-$) NK cells are immunoregulatory principally through cytokine production. Bright ($CD3^-CD56^{+bright}$) NK cells, which lack perforin granules, display homing receptors required for migration to secondary lymph nodes **[7].** A potential role for NK cells in viral hepatitis was first suggested by genetic studies that described a higher odds ratio of spontaneous HCV clearance **[20].**

In viral infections, NK cells exert rapid innate responses by exerting cytotoxicity against infected target cells and by releasing antiviral cytokines. By killing immature dendritic cells and secreting proinflammatory cytokines and chemokines, NK cells support the priming of T cells and orchestrate the recruitment of other immune cells to the site of infection **[33].** Some researchers suggested a close relationship between NK cell activation and disease outcome in HCV infection. Previous studies regarding phenotype and function of NK cells in patients with

viral hepatitis have revealed, in part, conflicting results.The objective of this study is to study the expression of natural killer cells in viral hepatitis patients and if there is difference in those patients when compared with normal population**[15,19]**.

Patients and methods

This study was carried out in the Clinical Pathology Department, Faculty of Medicine, Sohag University Hospital, on 76 patients who had been attending the outpatient clinic of the Tropical medicine and Gastroenterology Department, Qena Faculty of Medicine, South Valley University and known to have viral hepatitis infection. The ages of the viral hepatitis patients (as a group I) ranged from 21 to 65 years with a mean value of 42.13± 13.08(SD). 20 apparently healthy individuals of both sexes, their ages ranged from 17 to 64 years (as control group), with a mean value of 32.1 ± 10.33 (SD) .This study was approved by the Research and Ethical Committees at Faculty of Medicine, Sohag University. All patients were informed about the aim of this study and gave written consent.

All members of the study were subjected to the following:

I- Full history taking and clinical examination.

II- Laboratory investigations:

1. Complete blood count.
2. Prothrombin time and concentration.
3. Liver function tests.
4. Hepatitis C virus Abs and Hepatitis BsAg.
5. Assessment of naturalkiller cell markers CD3, CD16 and CD56expression.

Sample collection:

Venous blood samples were drawn from all patients and control groups under aseptic condition. Samples taken were divided into:

- 3 ml of venous blood were aseptically collected from each patient, dispensed into a tube containing K-ethylene diamine tetra-acetic acid (K-EDTA) at a concentration of 1.2 mg/ml, to be used for CBC and for flow cytometry.
- 1.8 ml of blood was collected to a vacutainer containing 0.2 ml citrated buffer for estimation of prothrombin time and concentration.
- Remnant of blood in a sterile plain vacutainer for other investigations,serum for sample analysis was obtained following centrifugation of whole blood after complete clot formation has taken place at 3000 rpm for 5 min.

Methods:

1- **Complete blood count (CBC):** was performed on the ABBOTT CELL-DYN 3700 automated hematology analyzer.

2- **Liver function tests:**Liver function tests were performed on Autoanalyzer Bechman Synchron CX5 system.

3- **Anti-hepatitis C virus antibodies (Anti-HCV Abs):**Anti-HCV Abs were performed on ARCHITECT i2000SR System using ARCHITECT Anti-HCV kits supplied by Abbott. Anti-HCV was estimated by a chemiluminescent microparticle immunoassay (CMIA) technology.

4- **Hepatitis B surface antigen(HBsAg):**HBsAg was performed on ARCHITECT i2000SR System using ARCHITECT HBsAg kits supplied by Abbott. HBsAg was estimated by a chemiluminescent microparticle immunoassay (CMIA) technology.

5- **Natural killer cell markers CD3, CD16 and CD56:**

FCM is the measurement of numerous cell properties (cytometry) as the cells move in single file resulting in light scattering. Antibodies specific for various cellular antigens can be labeled with different fluorochromes that can absorb and emit light, allowing simultaneous multicolor flow cytometric analysis of two or more cell-associated antigens.

Reagents:

1- Sheath fluid.
2- Phosphate buffered saline (PBS) (8.0 g/L NaCl, 0.2 g/L kcl, 1.15 g/L NaH2PO4 and 0.2 g kH2PO4) added to 100 mL of distilled water with pH adjusted at 7.3 ± 0.2.
3- Lysing solution (1.5 mmol/L NH4Cl, 100 mmol/L KHCO3 and 10 mmol/L tetra Na-EDTA) made up to 1 liter with distilled water, pH adjusted at 7.2.
4- Negative isotypic control (appropriately labelled according to the MoAbs used) for determining the non-specific binding of MoAbs.
5- MoAbs supplied by BD Bioscience, United states.

The panel of fluorescein isothiocyanate (FITC), phycoerythrin (PE) and Peridinin chlorophyll (PerCP) conjugated MoAbs was used for each sample to know the role of natural killer cells in viral hepatitis:

- T cell marker: CD3.
- Natural killer marker: CD56 and CD16.

***Procedure*:**

PB samples were processed within 24hours of collection. When this was not possible, the samples were left overnight at refrigerator.

- Blood was diluted with PBS so that TLC was adjusted between 5 and 10 x 10^3 / mm^3.
- For each sample, sets of tubes were labeled for all the MoAbs to be used, including 1 tube for the appropriate negative isotypic matched control MoAb.
- 50 µL of diluted samples were delivered in each tube.

- 5 µL of each MoAb as well as of the isotypic negative control MoAb were added to the respective tubes.
- The tubes were vortexed and incubated in the dark at room temperature for 15 minutes.
- 2 ml of PBS, as a wash buffer, were added to each tube and mixed thoroughly.
- The tubes were centrifuged at 3000 rpm for 5 minutes and the supernatant was discarded.
- 1.5 mL lysing solution was added to each tube.
- The tubes were vortexed and incubated for 5-10 minutes in the dark at room temperature.
- 2 ml PBS was added and the tubes were vortexed.
- The tubes were centrifuged at 5000 rpm for 5 minutes and the supernatant was discarded.
- Cells were suspended in 500 µL PBS to be ready for acquiring data by the FCM.
- Gating was done on the Natural Killer population based on forward and side scatter properties.

Results

This study was carried out on 76 patients who had been known to have viral hepatitis infection and 20 apparently healthy individuals as a control group. Clinical and hematological data of the studied patient and control groups are presented in (Table 1).

Table (1): Demographic and clinical data of patient and control groups

Items	Viral hepatitis (n =76)	Control (n =20)
Age		
Range	21-65 ys	17-64 ys
Mean ± SD	42.13 ± 13.08	32.1 ± 10.33
sex		
Male	55 (72.4%)	**12(60%)**
Female	21 (27.6%)	**8(40%)**
HCV Abs	**41 (54%)**	**0**
HBsAg	35 (46%)	**0**

Laboratory investigations:

1) Complete blood count: (Table 2)

Mean value of WBCs count, hemoglobin level and platelets revealed no statistically significant difference in group I when compared with control group (P> 0.05). Mean value of WBCs count, hemoglobin level and platelets revealed no statistically significant difference in HCV patients when compared with HBV patients (P >0.05).

2) Prothrombin time, Concentration and INR: (Table 3)

Mean value of INR revealed no statistically significant difference in group I, HCV patients and HBV patients when compared with control group (P > 0.05) respectively.

3) Liver function tests: (Table 4)

- **Alanine aminotransferase (ALT):**

Mean value of serum ALT revealed statistically significant elevation in viral hepatitis patient groups, HCV patients and HBV patients when compared with control group (P= 0.004 , 0.001, 0.001).With no statistically significant difference in HCV patients when compared with HBV patients (P > 0.05).

- **Aspartate aminotransferase (AST):**

Mean value of serum ALT revealed statistically significant elevation in viral hepatitis patient group, HCV patients and HBV patients when compared with control group(P<0.05).Mean value of serum AST revealed statistically significant elevation in HCV patients when compared with HBV patients (P<0.05).

- **Total serum bilirubin :**

The median value of total serum bilirubin showed statistically highly significant elevation in patient group, HCV patient, and significant elevation in HBV patients when compared with control group (P < 0.0001, P< 0.0001, P < 0.05). No statistically significant difference in HCV patients when compared with HBV patients (P > 0.05).

- **Serum Direct bilirubin:**

Statistically highly significant elevation of the median value of serum direct bilirubin was noted in patient group, HCV patients and significant elevation in HBV patients when compared with control group (P < 0.0001, P <0.0001 and P< 0.01) respectively) but no statistically significant difference in HCV patients when compared with HBV patients (P > 0.05).

- **Albumin (ALB):**

Mean value of serum albumin revealed no statistically significant difference in viral hepatitis patient group, HCV patients and HBV patients when compared with control group (P> 0.05) and no statistically significant difference in HCV patients when compared with HBV patients (P> 0.05).

4- Natural Killer cell markers (CD3, CD56&CD16): (Table 5)

Mean value of CD3 revealed highly statistically significant elevation in patients group, HCV patients and HBV patients when compared with control group (P < 0.0001) and no statistically significant between HCV patients when compared with HBV patients (P > 0.05).Mean value of CD56 revealed no statistically significant between patients group, HCV patients and HBV patients when compared with group II and between HCV patients and HBV patients (P > 0.05).

Mean value of $CD3^-$& $CD56^+$ and mean value of $CD3^-$& $CD16^+$ revealed statistically significant elevation between patients group, HCV patients and HBV patients when compared with control group ($P < 0.001$) and revealed no statistically significant difference between HCV patients and HBV patients ($P > 0.05$).

The Natural Killer cells($CD3^-$, $CD16^+$)& ($CD3^-$,$CD56^+$) percentage showed no statically significant correlation with ALT, AST, serum bilirubin, serum albumin or prothrombin time, concentration, INR and peripheral hemogram ($P > 0.05$).

Table 2: Peripheral hemogram in the studied groups.

Groups		HCVpatients (n =41)	HBVpatients (n =35)	Control (n =20)
Items				
WBCs (x 10^9/L)	Range	4.6 - 9.36	4.2 - 10.40	3.70 - 9.30
	Mean ±SD	6.84 ±1.37	5.47 ±1.77	6.12 ±1.51
Neutrophils	Range	30 – 75	34 – 64	31 – 66
	Mean ±SD	54.65± 11.77	52.2±9.5	51.25±10.33
Lymphocyte	Range	2 – 55	17 – 52	24 – 54
	Mean ±SD	32.85± 12.25	33.85 ±9.05	36.5± 9.07
Eosinophil	Range	2 – 8	1 –12	1 – 5
	Mean ±SD	3.65± 1.42	5.05±3.22	2.75±1.37
Monocyte	Range	4– 10	4 -10	5–10
	Mean ±SD	7.35 ± 1.87	7.9±1.68	7.8 ± 1.4
Basophile	Range	0.01 – 0.0 2	0.01 – 0.02	0.01 – 0.02
	Mean ±SD	0.20 ± 0.42	0.20 ± 0.42	0.20 ± 0.42
HGB (g/dl)	Range	12.20 -16.20	10.40 -16.60	10.40 -16.60
	Mean ±SD	13.78 ± 1.01	14.40 ± 1.05	13.16 ± 1.67
Platelets (x10^9/L)	Range	169 –495	127-321	154-424
	Mean ±SD	251.95 ± 76.66	207.25 ± 49.81	287.55 ± 72.72

Table 3: Prothrombin time, concentration and INR.

Group		HCV patients (n=41)	HBV patients (n = 35)	Control (n = 20)
Items				
PT(sec.)	Range	10.9-14.6	10.2 – 13.4	11 – 14
	Mean ±SD	12.05 ±0.98	11.67± 0.83	12.53 ± .84
PC(%)	Range	76 – 120.8	76.8– 117.9	75– 120.8
	Mean ±SD	94.63 ±10.80	97.29 ±10.83	99.74 ±13.25
INR	Range	0.9-1.19	0.9 – 1.19	0.9 – 1.19
	Mean ±SD	1.03±0.09	1.02±0.07	0.90 ±1.15

Table 4: Liver function tests in the studied groups.

Groups		HCV patients (n=41)	HBV patients (n=35)	Normal Control (n=20)
Items				
ALT (IU/L)	Range	14-108**	12 – 62**	9 – 39
	Mean ± SD	37.81±20.02	27.20 ±11.96	20.30±7.24
AST (IU/L)	Range	13– 94*	9.6 – 107*	5 – 37
	Mean ± SD	39.85±20.98	33.78 ±24.62	19.85±7.96
Total bilirubin (mg/dl)	Range Mean ± SD Median	0.8 - 4.8*** 1.9 ± 1.6 1.5	0.4 – 4.0 * 1.8 ± 1.4 1.3	0.2 – 1.0 0.6 ± 0.35 0.6
Direct bilirubin (mg/dl)	Range Mean ± SD Median	0.3 - 3.2*** 1 ± 0.8 0.735	0.09 – 3.1** 1.1 ± 1 1	0.2 – 0.4 0.6 ± 0.35 0.6
Albumin (g/dl)	Range	3.10- 4.40	3.20- 4 .0	3.50-5.20
	Mean ± SD	3.3 ± 0.85	3.83 ±0 .51	4.34 ± 0.56

* Significant (S) ($P < 0.05$); **Significant ($P < 0.01$); *** Highly Significant ($P < 0001$).

Table (5): Natural Killer cell markers (CD3, CD56&CD16).

		HCV patients	HBV patients	control	P value
CD3	Range	26-77.5	24.9-71.84	55.29-74.52	(HS)
	Mean ±SD	54.23± 16.09***	51.08 ±13.88***	65.92± 5.45	
CD56	Range	0.08-33	1.3-25	0.21-19.10	(NS)
	Mean ±SD	8.88± 6.18	10.47± 5.9	7.15± 4.45	
CD16	Range	0.1-37	2.3-26	0.06-15.18	
	Mean ±SD	9.3± 4.2	11. 7± 3.1	6.21± 2.12	(NS)
CD3-ve & CD56+ve	Range	4.43-30.38	0.44-23	0.00-15.10	
	Mean ±SD	13.06± 7.45**	9.83± 6.17**	6± 4.02	(S)
CD3-ve & C16+ve	Range	6.43-36.12	0.21-21	0.01-12.50	
	Mean ±SD	15.1± 6.25**	8.93± 5.77**	5± 3.82	(S)

***Highly significant (p< 0.001); **Significant (p < 0. 01). ; *Significant (p< 0. 05); Non-Significant (NS) (P > 0.05).

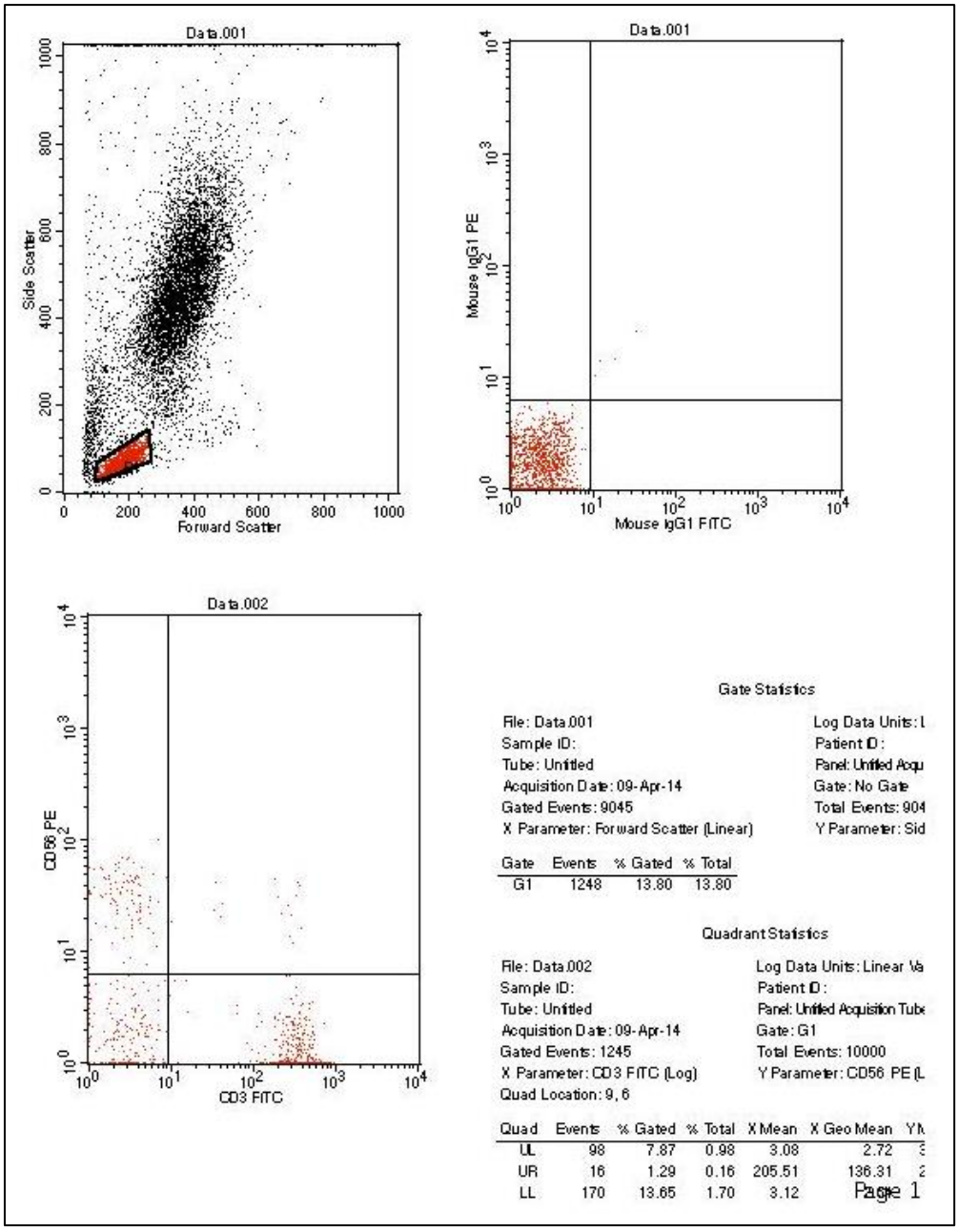

Fig.1 Flowcytometry result of HCV patient

Discussion

Viral hepatitis has emerged as a major public health problem throughout the world affecting several hundreds of millions of people. Viral hepatitis is a cause of considerable morbidity and mortality in the human population, both from acute infection and chronic sequelae which include, in the case of hepatitis B and C, chronic active hepatitis and cirrhosis. Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and is a leading cause of cancer-related death worldwide **[6]** and it is closely associated with hepatitis B, and at least in some regions of the world with hepatitis C virus More than 500 million individuals are chronic carriers of hepatitis B and hepatitis C viruses **[23].**

In this study, most of the patients were middle aged and these patients chosen early in the course of the disease which explained that there was no statistically significant difference in peripheral hemogram parameters and prothrombin time ,concentrations, INR and serum albumin in patients group, HCV and HBV patients when compared with control group which is consistent with some researchers who found that early in hepatitis thrombopoietin levels, prothrombin precursor protein and liver capacity of synthesizing clotting factors are still normal in the patients**[27].**

ALT & AST level showed significant increase in patients group when compared with control group in this study, there is statistically significant elevation of ALT in HCV patients when compared with HBV patients.This finding is in agreement with the finding of other investigators who found that liver enzyme abnormalities are frequently seen during the course of viral hepatitis **[12].**

Albumin level showed no statically significant difference in case group when compared with control group which can be explained as in early course of hepatitis before cirrhosis, there is no significant decrease in albumin level as the liver ability to synthesize the albumin is still preserved while a decline is expected late in chronic hepatitis, particularly in the presence of cirrhosis.

In this study, we found that there is increased percentage of T lymphocytes in peripheral blood in patients group when compared with healthy control group which noted from highly significant increase in $CD3^+$ lymphocytes in both HCV and HBV patients when compared with control group as virus-specific T-cell responses are able to successfully clear the virus in a subpopulation of patients, failure of these T-cell responses is associated with the development of viral persistence but the precise mechanisms of liver homing of virus-specific T cells are still unknown **[32].**

The NK cell population in the setting of viral hepatitis was identified by flow cytometry through the expression of CD56 and CD16 and the absence of CD3**[23].**

The percentageof different phenotypes of NK cells ($CD3^-$, $CD16^+$) & ($CD3^-$&$CD56^+$) showed statistically significant increase in patient group, HCV and HBV patients whencompared with healthy control group. This is in accordance with **[8, 28]** other researchers who found that hepatitis further increases the number of NK cells in the liver. This is can be explained by presence of Chemokines secreted by Kupffer cells recruit NK cells to the liver, and cytokines secreted by the Kupffer cells, liver sinusoidal endothelial cells, and T cells promote the survival of the recruited NK cells **[18].** Because of their enrichment in the liver and their ability to eliminate viral infections, NK cells may play key roles in the immune response against HCV infection**[1].** Some researchers **[23]** suggested a reshaping of the NK cell pool during hepatitis infections toward more $CD56^{bright}$ NK cells.

Natural killer cells are a major component of the innate immune system. Peripheral blood mononuclear cells (PBMCs) contain about 5%–15% of NK cells **[10]** and NK cells make up 30%–50% of the intrahepatic lymphocyte compartment **[14,23].**

Previous studies regarding phenotype and function of NK cells in patients with viral hepatitis have revealed, in part, conflicting results. As the state of NK cells associated with the fate of the viral infectionso NK cells have exhibited an activated phenotype in some studies **[3].**

In this study, the Natural Killer cells ($CD3^-$,$CD16^+$) & ($CD3^-$&$CD56^+$) percentage showed no statistically significant correlation with serum ALT, AST, total and direct bilirubin in patients group , HBV and HCV patients but **[34]** reported a positive correlation between NK cell counts and AST and ALT in Crimean–Congo hemorrhagic fever (CCHF) and also **Cho,[8]** reported that NK cell levels are directly associated with AST and ALT in HAV infection, but not in non viral hepatitis.

[29]Some researchers reported a correlation between the magnitude of T-cell response and the peripheral blood NK cell response in the acute phase of HCV infection, and **[21]** found that NK cells from patients who later cleared the infection have a greater antiviral effect in vitro than NK cells from patients who developed chronic HCV infection, an effect that has been associated with spontaneous recovery, from acute hepatitis C infection **[15].**

In chronic hepatitis B and C, a suppressed functionality of NK cells have been observed that potentially contributes to viral persistence **[11].** Yet other studies suggest increased cytolytic responses of NK cells and implicate a role for these cells in the pathogenesis of HBV and HCV infection **[36].**

Interestingly, the enhanced NK cell responses during interferon-α-based therapy of chronic hepatitis C indicate successful treatment **[35].**

Further research in the emerging field of liver-resident NK cells, and NK-cell-mediated memory functions and use of physiological models is important to increase our knowledgeof the immune surveillance in the liver and settle the controversies over the role and functional status of NK cells and may lead tonovel strategies for immunotherapy of hepatocellular carcinoma.

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دراسة بعض أنماط الخلايا الطبيعية القاتلة فى مرضى التهاب الكبد الفيروسى

شيرين فيليب عزيز& اشرف خضيرى محمد و على عبد الرحمن ومحمد هلال

قد يأتي التهاب الكبد الفيروسي فى صورة حادة (التهاب حديث , وبداية سريعة نسبيا) أو بشكل مزمن. حيث أن الأسباب الأكثر شيوعا للالتهاب الكبدى الفيروسى هى فيروس التهاب الكبد B وفيروس التهاب الكبد C. كما يوجد بالإضافة إلى فيروسات التهاب الكبد الاسمية فيروسات أخري والتي يمكن أيضا أن تسبب التهاب الكبد و تشمل الهربس البسيط , الفيروس المضخم للخلايا وفيروس ابشتاين بار أو الحمى الصفراء . توجد الخلية القاتلة الطبيعية (NK) بوفرة في الكبد وقد تكون سببا في حدوث ضرر للكبد في المرضى الذين يعانون من عدوى الفيروس الكبدى B المزمن . ومع ذلك يبقى دور الخلايا القاتلة الطبيعية في العدوى الحادة يحتاج إلى توضيح .

الهدف من الدراسة: دراسة بعض من دلالات التمايز للخلايا القاتلة الطبيعية عند مرضى التهاب الكبد المزمن والتي في الغالب من عدوى التهاب الكبدالفيروسى B , والتهاب الكبد الفيروسى C

تصميم الدراسة :المرضى :ستجرى هذه الدراسة على٣ مجموعات: المجموعة الأولى :تشمل مرضى الذين يعانون من عدوى التهاب الكبد ى B المزمن.المجموعة الثانية : تشمل مرضى الذين يعانون من عدوى التهاب الكبد الوبائي C المزمن. -المجموعةالثالثة : تشمل عشرة متطوعين أصحاء كمجموعة ضابطة . سيخضعون للاتي:أخذ التاريخ المرضى والفحص السريري الكامل وموجات فوق صوتية على البطن.-التحاليل المعمليةالتقليدية كالاتى:صورة دم - وظائف كبد -زمن وتركيزالبروثرومبين-الأجسام المضادة لفيروس الالتهاب الكبدىCواختبار مصلى لفيروس الالتهاب الكبدى ب (HBsAg)ومحددات التمايز ٣ (CD3) &(CD16)١٦ (CD56) ٥٦ .النظر الأخلاقية :تم مراجعةهذا البحث من قبل اللجنة العلميةالأخلاقية من مستشفي سوهاج الجامعي ,واخذ موافقه خطيه مسبقه من جميع الأشخاص سواء مرضى أو أصحاء.

النتائج: وقد وجد ان هناك زيادة كبيرة في نسبة المحددات المسئولة عن تنشيط الخلية القاتلة الطبيعية CD56 ٣&١٦CD, في مرضى التهاب الكبدى الفيروسى Bالمزمن و مرضى التهاب الكبد الفيروسي C الوبائي مقارنة مع الأفراد الأصحاء من خلال فحص الخلية القاتلة الطبيعية (NK) عن طريق تحليل التدفق الخلوي وقد لوحظ ان تغيرات انماط الخلايا القاتلة الطبيعية فىالتهابات الكبد الفيروسية B&C وهى تختلف فى التهاب الكبد الفيروسى بالنسبة لدرجة نشاط المرض وليس باختلاف الفيروس المسبب للمرض.