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EVALUATION OF THE IMPACT OF HLA-G 14BP POLYMORPHISM ON THE SUSCEPTIBILITY OF HEPATITIS C VIRUS INFECTION IN EGYPTIAN PATIENTS

By

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ABSTRACT

Background: Viral hepatitis was estimated to be the 7th leading cause of mortality globally. About half of this mortality is attributed to hepatitis C virus (HCV). Egypt is the most affected nation by HCV. Human Leucocytic Antigen-G (HLA-G) has an immunosupressor function. Many polymorphisms of HLA-G were reported. Most of those polymorphisms increase HLA-G expression, which increase the susceptibility to HCV infection.

Objective: To evaluate the HLA-G 14bp deletion polymorphism on the susceptibility to hepatitis C virus infection.

Patients and methods: The study was carried out on 90 subjects (30 females and 60 males) at Al Hussein University Hospital. Their age ranged from 27 to 70 years. They were classified into two groups: Group 1 (control group) included 30 normal healthy subjects (10 females and 20 males), aged 28-68 years and Group 2 (patient group) included 60 patients with HCV infection (20 females and 40 males), aged 27-70 years. All subjects of both groups were tested for detection of HLA-G 14bp deletion polymorphism.

Results: The HLA-G 14bp polymorphism was detected in 3 subjects of the control group (10%), while it was detected in 33 patients (54.9%) of the patient group. This revealed a significant increase of HLA-G 14bp deletion ($P < 0.05$) in patient group compared to control group. Conclusion: The HLA-G 14bp deletion polymorphism increased the susceptibility for HCV infection.

Keywords: HCV, HLA-G, susceptibility.

INTRODUCTION

The HCV has developed various strategies to protect itself from the host immune system. An effective one is up-regulation of non-classical Human Leucocytic Antigen-G (HLA-G) molecules through secretion of cytokines, as interleukin-10 *(Amiot et al., 2014a)*.

Human Leucocytic Antigen-G is a non-classical HLA class I molecule. It is known for its suppressive function and has 7 different isoforms. Differing from the classic HLA class I molecules, HLA-G is characterized by its restricted tissue distribution, low rate of polymorphism, and immunosuppressive properties *(Donadi et al; 2011)*.

Many polymorphisms occur mainly at the 5′ promotor region and 3′ untranslated regions (UTR) of HLA-G gene. The polymorphisms of the HLA-G gene lead to production of many isoforms and increased production of HLA-G which are implicated in many pathological conditions *(Rizzo et al., 2014b)*.

It has been shown that HLA-G isoforms promote regulatory T cells which suppress the proliferation and functions of T cells *(Du et al., 2011)*. It affects cooperation between B and T lymphocytes thus it inhibits proliferation and differentiation of B lymphocytes and their immunoglobulin secretion *(Naji et al., 2012)*. It also inhibits the function of neutrophils, key cells in host immune defense against pathogens and impairs phagocytosis and the respiratory burst of neutrophils responsible for reactive oxygen species production *(Baudhuin et al., 2013)*. HLA-G act as a negative regulator of the human immune response by several mechanisms. In addition, HLA-G inhibits the cytotoxic effects of T and Natural Killer (NK) cells, as well as the prevention of antigen recognition and anti-proliferative responses of CD4+ T cells *(Loustau et al., 2013)*. It is also demonstrated that HLA-G modulates adaptive and innate immunity by interacting with T or B lymphocytes, denderetic cells and NK cells *(Ramos et al., 2014)*.

Chronic hepatitis C Chinese patients were found to have a much higher plasma soluble HLA-G (sHLA-G) concentration than healthy subjects *(Weng et al., 2011)*. Also the up-regulation of soluble and membrane-bound HLA-G expression and its polymorphism was observed in Brazilian patients with the milder form of chronic hepatitis-C. The HLA-G expression has been, also, reported in the hepatocytes and biliary epithelial cells of the livers of patients with chronic hepatitis C *(de Oliveira Crispim et al., 2012)*. Thus, it was suggested that the HCV protects itself from the host immune system via up-regulating non-classical HLA-G molecules through secretion of cytokines, as interleukin-10. The polymorphism and increase of HLA-G levels help the HCV to persist and worsen its induced liver fibrosis *(Amiot et al., 2014b)*.

It was concluded that HLA-G polymorphism increases the susceptibility to HCV infection through its immunomodulatory role via many mechanisms. The HLA-G inhibits the cytolytic function of NK cells and cytotoxic T lymphocytes. Moreover it is also able to suppress the alloproliferative response of CD4 positive (CD4+) T cells and the proliferation of NK and T cells and shifting the immune response of T helper from T helper 1 (activator) to suppressor T helper 2 *(Catamo et al., 2017)*.

The aim of the present study was to evaluate the role of HLA-G 14bp deletion polymorphism on the susceptibility to HCV infection.

PATIENTS AND METHODS

This study was carried out on cases with HCV infection and apparently healthy control subjects. The patients were selected from those admitted to Al Hussein University Hospital. An informed consent was obtained from every subject and prior to their enrollment in this study.

The study was carried out on 90 subjects (30 females and 60 males). Their ages ranged from 27 to 70 years. They were classified into two groups:

Group 1 (control group) included 30 subjects (10 females and 20 males) serving as normal healthy control group (Proven by clinical and laboratory investigations). Their ages ranged from 28 to 68 years.

Group 2 (patient group) included 60 patients with HCV infection (20 females and 40 males). Their ages ranged from 27 to 70 years.

Inclusion criteria: Patients with HCV as diagnosed by ELISA and PCR.

Exclusion criteria: Patients with other hepatic diseases or HBV infections.

Subjects of both groups were subjected to the following studies:

1. Detailed history.
2. Laboratory investigation:

- HCV diagnostic workup by ELISA using fully automated analyzer, EVOLIS (Bio-Rad), and PCR, by PCR Thermal Cycler (Biometra).
- HLA-G 14 bp deletion polymorphism detection using conventional qualitative PCR. It was done by PCR Thermal Cycler (Biometra) usingCosmo PCR red Master Mix (Willowfort, Birmingham, UK).

Statistical Analysis: Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA).

RESULTS

There was no difference between sex of patient group [males (66.67) and females (33.33)] and control group [males (66.67) and females (33.33)].
There was, also, a non-significant difference in the age (P= 0.610) between the control group (Mean 48.87±SD11.51) and the patient (50.23±12.14) group **(Table 1)**.

The total number of HLA-G polymorphism (mutant and heterozygous deletions) was 3 subjects (10%) of the control group while it was detected in 33 patients (54.9%) of the patient group. This revealed a significant increase (P <0.05) in patient group compared to control group **(Table 1)**.

There was mutant deletion of HLA-G 14 bp in one subject (3.33%) of the control group while it was detected in 16 patients (26.6%) of the patient group. This revealed a significant increase (P <0.05) in patient group compared to control group **(Table 1)**.

There was heterozygous deletion of HLA-G 14 bp in 2 subjects (6.67%) of the control group while it was detected in 17 patients (28.3%) of the patient group. This revealed a significant increase (P <0.05) in patient group compared to control group **(Table 1)**.

The HLA-G 14 bp polymorphism was not detected (wild) in 27 subjects (90.1%) of the control group while it was not detected in 27 patients (45.1%) of the patient group. This revealed a significant decrease (P <0.05) in patient group compared to control group **(Table 1)**.

Table (1): Comparison between the control group and patient group as regards age and HLA-G 14 bp polymorphism

Groups / Parameters		Control (N=30)		Patients (N=60)		
		Mean and Standard Deviation		Mean and Standard Deviation		P value
Age in years		48.87 ± 11.51		50.23 ± 12.14		0.610
HLA-G 14 pb deletion		count	% of total	count	% of total	
	Mutant	1	3.33	16	26.6	<0.05
	Heterozygous	2	6.67	17	28.3	
	Total polymorphism	3	10	33	54.9	
	Wild	27	90.1	27	45.1	

DISCUSSION

In the present study, the HLA-G 14bp deletion polymorphism was detected in 54.9% of the patient group while it was detected in only 10% of the control group. There was a significant increase in the polymorphism in the patient group compared to the control group. Thus, it could be suggested that the HLA-G 14bp deletion polymorphism has increased the susceptibility to HCV infection and chronicity.

This is in agreement with the findings of *Amiot et al.(2014a)* who reported that HLA-G polymorphisms interferes with the immune response to infections, through increased expression of HLA-G, and increases the susceptibility to HCV infection. *Donadi et al. (2011), Rizzo et al. (2012), Martelli-Palomino et al. (2013), Rizzo et al. (2014a)* reported that the HLA-G 14 bp deletion polymorphism was associated with high expression of HLA-G. In addition, *da Silva et al. (2014)* reported that increased frequency of HLA-G14 bp polymophisms increase the susceptibility to HCV infection. Moreover, *Amiot et al. (2014b)* suggested that HLA-G polymorphisms result in the increase of its expression which helps the HCV to persist.

This was supported by the findings of *Weng et al. (2011)* who found an increased plasma HLA-G levels associated with chronic HCV infection. Also, *de Oliveira Crispim et al. (2012)* found an increase in HLA-G hepatocyte expression in HCV-infected liver specimens. In addition, *Wolf et al. (2020)* who concluded that HLA-G polymorphisms increase the susceptibility for hepatitis B infection and chronicity.

Carosella et al. (2015) reported that HLA-G is an immune checkpoint molecule that inhibits the function of immunocompetent cells such as the cytotoxic activity of NK and T CD8+ lymphocytes, through the binding to inhibitory receptors. Also, *Catamo et al. (2017)* concluded that HLA-G polymorphisms increase the susceptibility to HCV infection through up-regulation of HLA-G expression.

On the other hand, in a meta-analysis study, *Lv et al. (2018)* demonstrated that HLA-G 14-bp Ins/Del polymorphisms may exert no influence on susceptibility to viruses. However, they added that, due to the limited number of studies examined, the results from that meta-analysis may be inconclusive.

CONCLUSION

The HLA-G 14bp deletion polymorphism increased the susceptibility for HCV infection. Therefore, the HLA-G 14bp deletion polymorphism was considered an additional risk factor for HCV infection.

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دراسة تأثير التغير في مستضد الكريات البيضاء البشرية HLA-G14bp على قابلية العدوى بالالتهاب الكبدي الوبائي (سي) في المرضى المصريين

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خلفيـــة البحـــث: يُقـــدر التهـــاب الكبـــد الفيروســـي بأنـــه الســـبب الســـابع للوفيـــات علـــى مســـتوى العـــالم. يُعـــزى حـــوالي نصـــف هـــذه الوفيـــات إلـــى فيـــروس التهـــاب الكبـــد الوبـــائي ســـي. تعتبـــر مصـــر هـــي الدولـــة الأكثـــر تضـــررا مـــن هـــذا الفيـــروس. مستضـــد الكريـــات البيضـــاء البشـــرية G لـــه دور فـــي تثبـــيط المناعـــة. تـــم اكتشـــاف العديـــد مـــن التحـــورات لهـــذا الجـــين و معظمهـــا تزيـــد مـــن قابلية الاصابة بالتهاب الكبد الوبائي سي.

الهـــدف مـــن البحـــث: تقيـــيم تحـــور مستضـــد الكريـــات البيضـــاء البشـــرية G على القابلية للإصابة بفيروس الالتهاب الكبدى سى.

المرضـــي و طـــرق البحـــث: أجريـــت الدراســـة علـــى 90 شخصًـــا (30 إنثـــى و 60 ذكـــرًا) تراوحـــت اعمـــارهم بـــين 27 و70 ســـنة وقـــد تـــم تصـــنيفهم إلـــى مجموعتين:

المجموعـــة الأولـــى (المجموعـــة الضـــابطة) تضـــمنت 30 شخصًـــا يتمتعـــون بصـــحة جيـــدة (10 إنـــاث و 20 ذكـــرًا) تراوحـــت اعمـــارهم بـــين 28 و68 سنة.

المجموعـــة الثانيـــة (مجموعـــة المرضـــى) تضـــمنت 60 مريضـــا مصـــابين بفيـــروس الالتهـــاب الكبدســـى (20 إنثـــى و 40 ذكـــرا) تراوحـــت اعمـــارهم بين 27 و70 سنة.

وقد تم اختبار جميع الاشخاص لكلتا المجموعتين لاكتشاف تحور حذف مستضد الكريات البيضاء البشرية G 14 bp.

نتائج البحث: اظهرت النتائج ان عدد التحور تم اكتشافه فى 3 افراد (10%) فى المجموعة الضابطة بينما تم اكتشافه فى 33 مريضا فى مجموعة المرضى (54.9%) وهذا يوضح ان هناك زيادة ذات دلالة احصائية فى هذا التحور فى مجموعة المرضى مقارنة بالمجموعة الضابطة.

الاستنتاج: تحور حذف مستضد الكريات البيضاء البشرية G 14 bp يزيد من القابلية للاصابة بالفيروس الكبدى-سى.

الكلمات الدالة: إلتهاب الكبد الوبائي سي، مستضد الكريات البيضاء البشرية G، قابلية.

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