INTERLEUKIN-12 LEVEL AND ITS RELATION TO HEPATITIS B VIRUS VACCINE RESPONSE IN HEMODIALYSIS PATIENTS

By

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ABSTRACT

Background: Patients with chronic kidney disease on hemodialysis are considered a vulnerable group. Due to the hemodialysis process, they are subjected to blood transmitted infections like hepatitis B virus. Uremia alters their immune system, which alters the response to vaccination against hepatitis B virus.

Objective: Detection of the relationship between interleukin-12 (IL-12) level and, the response to hepatitis B vaccine in chronic kidney disease patients on hemodialysis.

Patients and methods: This study was conducted in Sayed Galal Hospital among 60 chronic kidney disease patients on regular hemodialysis, and 25 healthy workers in the dialysis unit as a control group. Both groups were vaccinated against hepatitis B virus using Energix vaccine with full 4 doses. All subjects were subjected to detailed history taking, full medical examination, and some laboratory investigations. Data obtained from these procedures were then analyzed using SPSS program.

Results: Response to hepatitis B vaccine was higher among control group. Interleukin-12 level was also higher among the control. However, in both the case and control group, there was a positive association between the level of interleukin-12 and level of hepatitis B surface antibodies (HBsAb) denoting better response.

Conclusion: high levels of interleukin-12 were positively associated with better response to hepatitis B vaccine in patients with chronic kidney disease on hemodialysis and can be used as a predictor of response to vaccination.

Key words: Chronic kidney disease, hemodialysis, hepatitis B vaccine, interleukin-12.

INTRODUCTION

Hepatitis B infection is a threat to hemodialysis patients. Since these patients have altered immune response and at higher risk of developing chronicity with all its consequences like cirrhosis, liver cell failure and even cancer, protection of this population is a critical issue **(Somi et al., 2012).**

According to the recommendations of the Advisory Committee on Immunization Practices a dose of 40 mcg of recombinant hepatitis B vaccine is routinely administered to hemodialysis patients at 0, 1, 2, and 6 months **(CDC, 2011).** This

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regimen was thought to be protective for those patients. However, it was found that there are variations in the response of the vaccine **(Lin et al., 2012).**

Older age, male gender, smoking, obesity and diabetes were found to be associated with lower sero-conversion to the vaccine **(Bandaru et al., 2013; Ayub et al., 2014; Dede Sit et al., 2015 and Khedmat et al., 2016).** Some studies found that there is a relationship between efficient hemodialysis and production of anti-hepatitis B antibodies post vaccination in hemodialysis patients **(Ibrahim et al., 2006).** Similarly, better sero-conversion rate was found when vaccinating chronic kidney disease patients before they become hemodialysis dependent **(Gregozweska et al., 2012)**.

Interleukin -12 (IL-12) is a heterodimer cytokine with potent regulatory functions **(Méndez-Samperio, 2010).** It was found to play a role in the clearance of hepatitis B virus (**Rizvi et al., 2012**), used as vaccination therapy **(Zeng Z et al.,2013)** , and polymorphism is associated with development of hepatitis B antibodies after vaccination and natural infection **(Grzegorzewska et al., 2012 and 2013).**

SUBJECTS AND METHODS

This is a case control study conducted in Sayed Galal Nephrology and Dialysis unit, Al- Azhar University. It included 60 chronic kidney disease patients on hemodialysis as patients group and 25 healthy workers at the dialysis unit of matched age and sex as control group. All subjects were negative for all serological markers of hepatitis C and hepatitis B infection.

We prospectively studied the evolution of hepatitis B surface antibodies after primary vaccination. (4 doses; Energix B vaccine, 40 mcg intramuscular in the deltoid region at 0,1,2,and 6 months for patients group and 3 doses ; Energix B vaccine, 20 mcg intramuscular in the deltoid region at 0, 1 and 6 months for the control group).

The mean duration of hemodialysis therapy was 3.8 ± 0.6 years, range (1-5) years. The patients group were on hemodialysis therapy trice a week using hollow fiber dialyzer ($1.3 -1.6m^2$) against standard bicarbonate dialysate. Blood access was arterio-venous fistula, Blood and dialysate flow rates were 300&500 mL/min. respectively. During dialysis, the patients received intermittent hepariniza-tion every hour with 5000-10000 units as maximum dose. The study protocol was approved by Al Azhar University ethics committee and written consent were obtained from all subjects.

To assess the response to the vaccine, we measured hepatitis B surface antibodies seven to twelve weeks after the last vaccine dose in both groups, using ELISA technique. Protection against hepatitis B infection is achieved if HBs Ab titre exceeds 10 IU/L. Non-response was considered if HBsAb titre is less than 10IU/L, poor response if HBs Ab titre between 10-100 IU/L and good response if HBs Ab titre more than 100IU/L.

Interleukin -12 titer was extracted from venous blood anti-coagulated with ethylene diamine tetra acetic acid (EDTA). The cells were lysed by incubation with proteinase K (Sigma Chemicals, Deisenhofen, Germany) for 10 minutes at 56°C. The DNA was analyzed

by highly specific ARMS-PCR (amplification refractory mutation system). This protocol is based on a polymerase chain reaction (PCR) using primers ending 3' with the complementary base for the single polymorphic base in the promoter sequence. Stringency of reaction parameters assured that a PCR product was only formed when the 3' base matches.

Statistical analysis:

The results were summarized as mean ± standard deviation (SD) for quantitative variables. Qualitative variables were presented as number and percent. Analysis of variance (ANOVA) was used to test the significance of different values between responders and non-responders. Pearson correlation was performed to assess the relation between Interleukin-12 and response to hepatitis B vaccine. P-value <0.05 was considered statistically significant. All statistical analysis was done using SPSS version 21.

RESULTS

Both cases and control were properly matched for age and sex. Cases group had significantly lower levels of hemoglobin and albumin and higher liver enzymes alanine transaminase (ALT). There was no statistical significance in their anti - hepatitis B surface antibody titre. Interleukin-12 level (IL-12) was higher among control group. However, this result was borderline significant (Table 1).

Table (1): Basic characteristics of studied groups.

Groups / Parameters	Cases (Mean ± SD)	Control (Mean ± SD)	P -value
Age (years)	44.4(± 6.7)	43.9(± 6.9)	0.2
Sex (No. & %) Male Female	33 (55%) 27(45%)	15 (56%) 10 (44%)	0.4
Hemoglobin (g/dl)	10.27(± 1.2)	13.52(± 1.0)	0.00
ALT (U/L)	32.10(± 12.2)	23.68(± 9.6)	0.00
Albumin (g/dl)	3.76 (± 0.4)	4.23(± 0.3)	0.00
HBs Ab (titre)	99.47(± 59.7)	89.46 (± 45.9)	0.4
IL-12 (Pg/ml)	63.8 (± 25.3)	74.86 (± 23.7)	0.06

Responders were significantly younger, with fewer years on hemodialysis. Creatinine level was significantly higher among non-responders. Both good and poor responders have significantly higher interleukin-12 compared to non-responders (Table 2).

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Table(2): Table (2): Basic characteristics and laboratory parameters of good, poor and non-responders among patient group.

Responders / Parameters	Good responders (No. 36)	Poor responders (No. 17)	Non responders (No. 7)	P –value
Age	45 ±10.2	45 ±6.3	55 ±7.9	0.01
Body mass index	26 ± 3.8	27 ±4.5	27 ±4.1	0.16
Years on hemodialysis	3.4 ±1.4	4 ± 1.6	5.6 ± 1.7	0.00
Blood Urea Nitrogen	46.3± 22	35.4± 20	47.3± 25	0.1
Creatinine	5.3± 3.1	3.1± 2.1	6.3± 3.4	0.00
Hemoglobin (g/dl)	11.1± 1.9	11.5± 1.8	10.9± 1.9	0.6
Albumin (g/dl)	3.8± 0.4	3.9± 0.4	4.1± 0.2	0.3
HBs Ab titre	138± 6.3	136± 8.1	140± 0.2	0.00
IL-12 (Pg/ml)	82.7± 15.2	51.4± 20.8	32.± 12.2	0.00

There was no statistical difference in the rate of response to hepatitis B vaccine between cases and control. Response to hepatitis B vaccine was determined by anti-hepatitis B antibody titre. Individuals with HBs Abtitre $\geq$ 100 considered good responders, while those with Hbs Ab titre 10-100 were poor responders and individuals with titre $\leq$ 10 are non-responders (Fig.1).
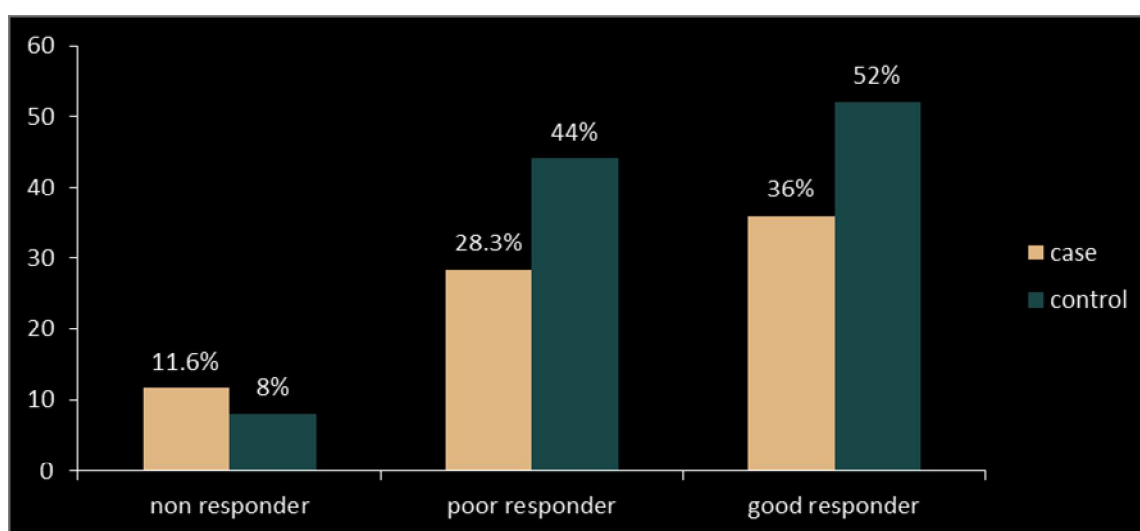
Figure (1): Rate of response to Hepatitis B vaccine among cases and control.

There was a positive correlation between hepatitis B surface antibody titre and Interleukin -12 (Figure 2).
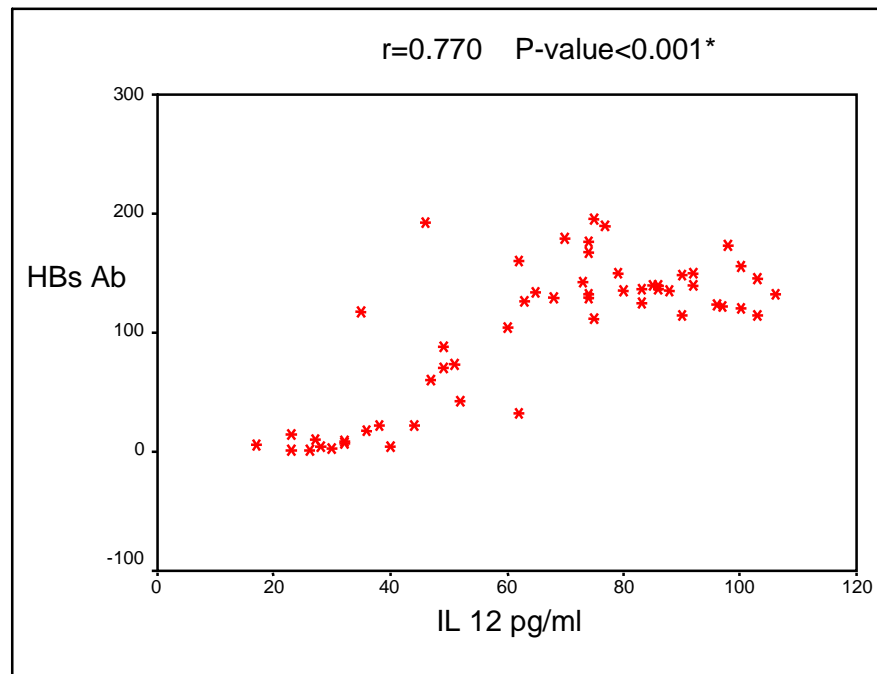

Figure (2): Scatter plot graph of correlation between the hepatitis Bsurface antibody titre and Interleukin-12.

DISCUSSION

Hemodialysis patients included in this study showed adequate response to hepatitis B vaccine where 60% of them were good responders and 28% were poor responders. These results were supported by other studies that found adequate post vaccination seroconversion among chronic kidney disease patients on hemodialysis, **(Ibrahim et al., 2006, Ouzoni et al., 2007 and El Saran et al., 2014).**

Among the patients group, responders were significantly younger than non-responders. This supported results of previous studies which stated that the older the age of the patient the poorer is the response to the vaccine **(Ibrahim et al., 2006 and Al Saran et al., 2014).**

Shorter duration on hemodialysis was positively associated with response to the vaccine and development of hepatitis B surface antibodies among hemodialysis patients in this study. Other studies suggested that duration on hemodialysis has no effect on the response to the vaccine **(Ibrahim et al., 2006 and El Saran et al., 2014)**. Another study found that the shorter the duration on hemodialysis the poorer the response to the vaccine **(Grzegrzewska et al., 2012)**. Therefore, no solid evidence in this issue was available. However, in the current study, age of responder and efficiency of hemodialysis might have been confounders.

There was no statistical difference between the body mass index of responders and non-responders in this

study. These results were consistent with the results of other studies which found no statistical difference between responders and non- responders **(Ibrahim et al., 2006 and Al Saran et al., 2014).** On the contrary, the results of the study conducted by Ouzoni et al. (2007) show significant difference in the body mass index of responders and non-responders, and patients with higher body mass index are poor responders to the vaccine.

Among studied laboratory parameters, serum creatinine was significantly lower among responders. This was consistent with results of another study conducted on hemodialysis patients, and found that patienst with creatinine level <4 mg/dl had higher anti hepatitis B surface antibodies (HBs Ab) post vaccination, compared to those with higher creatnine levels **(Ghadiani et al., 2012).** On the other hand, serum albumin and hemoglobin levels had no significant association with development of hepatitis B antibodies following vaccination. Other researchers concluded similar results regarding albumin and hemoglobin. **(Ouzouni et al., 2007, Behnam et al., 2011, Lin et al., 2012, Asfar et al., 2013 and El Saran et al., 2014)**.

In this study there was no statistical significance in the level of IL-12 in both cases and control. On the contrary, a study that compared IL-12 in chronic kidney disease not on hemodialysis, hemodialysis patients and healthy control, found higher IL-12 in both chronic kidney patients and hemodialysis patients compared to control group **(Yong et al., 2013)**.

In the current study, positive association was found between IL-12 and development of hepatitis B antibodies post vacci-nation. Responders had significantly higher IL-12. There was a positive correlation between IL-12 level and anti - hepatitis B surface antibody titre. Other studies found positive association between IL-12 and response to the vaccine **(Grzegorzewska et al., 2012 and Grzegorzewska et al., 2013**).

CONCLUSION

Hemodialysis patients were vulnerable group, at high risk of hepatitis B infection. Immunization against hepatitis B was the best preventive measures. There were factors that affect response of hemodialysis patients to the vaccine like age, hemodialysis duration and stage of chronic kidney disease. Interleukin-12 was associated with better response to the vaccine in hemodialysis patients.

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العلاقة بين وجود الإنترلوكين ١٢ فى الدم و إستجابة مرضى الفشل الكلوى المعاشين على الإستصفاء الدموى للتطعيم ضد فيروس الكبد الوبائى (ب)

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قسمى الأمراض الباطنة١ و الباثولوجيا الإكلينيكية٢

خلفية البحث: تعد الإصابة بفيروس الكبد الوبائى (ب) من أهم المخاطر التى يتعرض لها مرضى الفشل الكلوى المزمن المعاشين على الإستصفاء الدموى المتكرر ، حيث أنهم يتعرضون بشكل دائم للدم و مشتقاته التى قد تكون محملة بفيروس الكبد الوبائى (ب).من هنا كانت أهمية وجوب تطعيم كل مرضى الفشل الكلوى المعاشين على الإستصفاء الدموى ضد الإلتهاب الكبدى الوبائى (ب) مما يحد من إنتشار العدوى ، وقد أثبتت الدراسات أن الفشل الكلوى يسبب تغيرا فى عمل الجهاز المناعى، مما يؤدى إلى إختلال فى إستجابة المرضى للمصل. كما أن هناك عوامل أخرى تؤدى إلى إختلال إستجابة الجهاز المناعى لمرضى الفشل الكلوى للتطعيم مثل تقدم السن ، والنوع، والسمنة، و سوء التغذية.

الهدف من البحث: دراسة العلاقة بين مستوى الإنترلوكين ١٢ فى الدم و إستجابة المرضى للتطعيم ضد فيروس الكبد الوبائى (ب) عند مرضى الفشل الكلوى المعاشين على الإستصفاء الدموى، و مقارنتهم بالأصحاء من حيث الإستجابة للتطعيم و إنتاج الإنترلوكين ١٢.

المرضى و طرق البحث: تمت دراسة ستين مريضا مصابين بالفشل الكلوى المزمن و يعالجون بالإستصفاء الدموى كعينة بحث، و خمسة و عشرين عاملا بوحدة الغسيل الكلوى كعينة ضابطة. و تم مراجعة تاريخهم المرضى و إخضاعهم للفحص الطبى، و أخذ عينة دم لعمل بعض التحاليل.

النتائج: وجدت علاقة بين مستوى الإنترلوكين ١٢ والإستجابة للتطعيم ضد فيروس الكبد الوبائى (ب)، فكلما زادت نسبة الإنترلوكين ١٢ كلما تحسنت الإستجابة للمصل فى مرضى الفشل الكلوى كما فى الأصحاء. و قد كشفت الدراسة عن وجود بعض العوامل المؤثرة على الإستجابة للتطعيم لدى مرضى الفشل الكلوى مثل السن و نسبة الكرياتيين بالدم و عدد سنوات العلاج بالإستصفاء الدموى.

الخلاصة: هناك علاقة طردية بين مستوى الإنترلوكين١٢ فى الدم و الاستجابة للتطعيم ضد فيروس الكبد الوبائى (ب). لذلك، يعد إرتفاع نسبة الإنترلوكين١٢ فى الدم مؤشرا إيجابيا لإستجابة مرض الفشل الكلوى المعاشين على الإستصفاء الدموى للتطعيم ضد فيروس الكبد الوبائى (ب).