



## **Requirements for manuscripts submitted to the AL- Qadisiah Medical Journal**

**These requirements are adopted from the " uniform requirements for Manuscripts (URM) submitted to biomedical Journal by the International Committee of Medical Editors "**

**1\_ The Qad . Med. J. Prefers that all manuscripts be submitted on CD, along with the original typed man script plus 3 additional copies.**

**2 – The manuscripts should be types double spaced, including the title page, abstract, acknowledgment, references, tables and legends. Use only one side of each page. Leave margins of at least 25 mm (1 inch) on each side.**

**3- Articles will be accepted either in Arabic or English.**

**4- The title page should be submitted in Arabic and in English.**

**5- Illustration should be submitted on separate paper. The measurement of each should not exceed the size of a standard A4 page.**

**6- The abstract should be structured, submitted both in English and in Arabic the length of each of the abstracts should not exceed 250 words.**

**7- Articles should be no longer than 10 double – spaced pages including references.**

**8- Case reports should be no more than 3 pages including references.**

**9- Use Arabic numbers in all articles, both in English and Arabic (1, 2, 3...).**

**10- Articles previously published elsewhere are not acceptable.**

**AL- Qadisiah medical Journal A medical journal encompassing all medical specialization issued Twice year**

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## **Immunological assessment of hemodialysis patients in Al-Diwaniya and Al-Najaf governorates**

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الخلاصة

اجريت الدراسة الحالية للتحري عن الحالة المناعية لمجموعة خاصة من المرضى الذين تجرى لهم عملية الديليزة الدموية (الغسل الكلوي) بسبب وصولهم الى ما يسمى المرحلة النهائية (متأخرة) لمرضى الكلى (الفشل الكلوي= ESRD). شملت الدراسة مجموعة من المرضى (عدد=82) الذين يراجعون وحدة الديليزة الدموية في مستشفى الديوانية التعليمي و مستشفى الحكيم التعليمي / النجف الأشرف خلال الفترة مابين شهر تشرين الاول لسنة 2008 وشهر اب لسنة 2009. كما شملت الدراسة مجموعتين ضابطين (سيطرة) شملت الاولى مرضى مصابين بامراض بالجهاز البولي ولكن ليس العجز الكلوي وهم (10) مرضى ، و(22) مريض مصاب بالتهاب الكبد الفايروسي B أو C، والثانية ضمت (50) شخصا من الاصحاء عيانيا. جميع افراد الدراسة شملوا باستمارة المعلومات العامة المعدة لهذا الغرض.

جمعت عينات الدم من جميع افراد الدراسة وقسمت الى جزئين ؛ الاول لفحوص صورة الدم و الثاني (المصل) للفحوصات البايوكيميائية والمصلية والتي شملت قياس مستويات الانترفيرون كما والانترولوكين 8 (IL-8) باستخدام فحص الاليزا. كان تركيز الانترفيرون كما والانترولوكين 8- (pg/ml) بين المجاميع الاربع التي درست. ظهر الانترفيرون كما بمعدل (صفر) في مرضى العجز الكلوي ومجموعة مرضى الجهاز البولي، في حين كان معمله (0.1)، (0.05) في مجموعة التهاب الكبد الفايروسي ومجموعة الاصحاء على التوالي. وكان الاختلاف معنويا من الناحية الاحصائية بين المجموعة الاولى والثالثة فقط. وكان الاختلاف في تركيز IL-8 اكثر اهمية من الناحية الاحصائية حيث ظهر بمعدلات تركيز (24.1)، (23.7)، (86.9)، (104.1) في المجاميع الاربعه على التوالي.

اكدت نتائج هذه الدراسة حالة الانهيار المناعي في مرضى العجز الكلوي، والتي تزداد مع عمر المرض ومع ازدياد مرات الغسل الكلوي الاسبوعية، اضافة الى ظهور الانترفيرون كما والانترولوكين 8- كعوامل تفريقية جيدة بين المجاميع الاربعه. اوصت الدراسة باجراء دراسات مستقبلية اوسع تشمل عوامل مناعية اخرى.

### **Abstract**

The present study was carried out to investigate the immunological status of special category of patients who were ongoing Hemodialysis process as they at End-Stage Renal Disease (ESRD). The study group (patients) included were 82 individuals who were regularly attending HD units at AL-Diwaniya Teaching Hospital and AL-Hakeem Teaching Hospital (AL-Najaf AL-Ashraf) / Iraq during the period Nov.2008 - Aug.2009.

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Another two control groups; the 1<sup>st</sup> (n=50) who were apparently healthy and the 2<sup>nd</sup> (n=32) patients with UTI other than ESRD. They all subjected to a questionnaire form regarding many general variables. Blood samples collected and each divided into two aliquots; the 1<sup>st</sup> for hematology and the second (serum) for biochemical and serology tests including IFN- $\gamma$  and IL-8 assays using ELISA. The interesting results those of serum IFN- $\gamma$ , IL-8(pg/ml) and CRP(mg/ml) among the three study groups; the median concentration of IFN- $\gamma$  in HD patients and renal disease (not failure) was zero, while it was 0.05 in healthy control. However, the only significant difference was between the first and third groups. Interleukin -8 has seem to be more significant different as it has had median concentration of, 24.1, 23.7, and 104.1 in the three groups above, respectively.

### Introduction

End -Stage Renal Disease (ESRD) is a growing problem worldwide and renal replacement therapy is increasingly exerting pressure on health systems<sup>(1)</sup>. The situation is particularly serious in developing countries where health resources are limited. Patients with (ESRD) are normally subjected to regular hemodialysis (HD). Numerous research studies on the synthesis and the release of proinflammatory cytokines IL-1 $\beta$ , IL-2, IL-6, IL-8 and TNF- $\alpha$  in patients with chronic renal failure on maintenance hemodialysis provide contradictory data. Although some of these studies demonstrated increased serum levels of the proinflammatory cytokines prior to and in the course of hemodialysis, other studies indicated that cellular activation and cytokine synthesis is only transient and the increase of the serum levels is rather moderate<sup>(2,3,4,5,6,7,8)</sup>.

Prevalence of chronic kidney diseases (CKD) is continuously increasing along with hypertension and diabetes<sup>(9,10)</sup>, occurred in 30% in elderly persons<sup>(10)</sup>, and classified according to the level of kidney function<sup>(11)</sup>. It is also contributed to immunodeficiency, and infection are the second most common cause of death after vascular disease among dialysis patients<sup>(12,13,14)</sup>.

### Materials and Methods

Eighty-two (males=46, females=36) Hemodialysis (HD) patients from AL-Diwaniay hospital for teaching/unit of hemodialysis and AL-Hakeem hospital (in AL-Najaf) / unit of hemodialysis. All were either regularly attended or attended for the first time at hemodialysis unit. Control group was composed of two subgroups ; the 1<sup>st</sup>, 50 individuals who were apparently healthy , the 2<sup>nd</sup>, 32 patients with renal and hepatitis disease . The specimens were blood samples collected from all members of the study groups.

The **Reflotron Plus** (Reflotron® Roche Diagnostics GmbH/ Germany) strips was used to determine serum; urea, creatinine, Ca<sup>+2</sup> , TSB, and GPT. CRP-latex test kit (Spinreact / Spain) used to assess serum CRP.

Human IL-8 /NAP-1 (ELISA kit:BMS204/3. Mender MedSystems GmbH

Austria , Europe). IFN $\gamma$  kit - (ELISA IM1743:Immunotech / Marseille Cedex 9 /France).

### Results and Discussion

The hemodialysis patients are ranged in age from 11 to 92 years, A standardized form is used to collect data regarding age, sex, duration of dialysis, the number of blood transfusions, the weekly frequency of dialysis, causes of chronic renal failure ,such as family history of renal failure and history of renal transplantation.

Table (1), shows our findings in this respect which are consistent with various reports as males were represented by a higher percentage (56.1%) compared to females population (43.9%). The majority of ESRD patients in almost all countries are males rather than females<sup>(15)</sup> . Depner 2003, suggests that women are with a better response to clearance of uremia toxins, compared to men<sup>(16)</sup>. In our study the different in this percentage is not reported but it may be referred to many causes such as the family or congenital history (e.g. stone of kidney) was more affected on the males than the females, and the disease that lead to renal failure (e.g. hypertension, diabetes ) are more common in males than females. This result in agreement with data from other countries<sup>(1,17,18)</sup>. In the group of the renal disease not failure the gender is 50% males and 50% females, while the group of hepatitis are 9.1%females,

and 90.9% males. But the group of healthy controls unknown the types of gender because the blood samples were collected from the blood donors.

**Table 1: Gender distribution in 3 study groups.**

Gender	Study group							
	Cases (Hemodialysis patients)		Cases controls (Renal disease, not failure)		Cases controls (Hepatitis, no renal disease)		Healthy control	
	N	%	N	%	N	%	N	%
Females	36	43.9	5	50	2	9.1	8	16
Males	46	56.1	5	50	20	90.9	42	84
Total	82	100	10	100	22	100	50	100

Among many factors studied as function of the age of hemodialysis, table 2 shows that the serum CRP, total WBC count, neutrophile and lymphocyte count were significantly affected, supporting the immune-suppression idea in such group of patients referred to by many authors.

For INF-gamma and IL-8 assays, individual in whom the level of these two mediators have estimated, they were 45 of HD patients, 6 of renal disease not failure, 22 of hepatitis patients and 15 of healthy control group, that is for the limited number of tests supported by the kit (table 3).

Table 2: The effect of age of hemodialysis on different immunological parameters.

Parameters	Duration of hemodialysis in years-quartiles			P-Value
	First (Lowest) quartile ( $\leq$ 1 month)	Inner-quartile range ( $\leq$ 1.5 years)	Fourth (highest) quartile ( $>$ 1.5 year)	
<b>Serum Interferone gamma conc (pg/ml)</b>				<b>&gt; 0.05</b>
Median	-	0	0	
Mean rank	-	21.38	25.21	
N	0	26	19	
<b>Serum IL8 conc (pg/ml)</b>				<b>&gt; 0.05</b>
Median	-	24.1	24.1	
Mean rank	-	23	23	
N	0	26	19	
<b>Serum CRP (mg/L)</b>				<b>&lt; 0.05</b>
Median	12	0	0	
Mean rank	50.09	40.27	29.87	
N	22	39	19	
<b>Blood total WBC count (x10<sup>9</sup>/L)</b>				<b>&lt; 0.05</b>
Median	6	4.2	3.6	
Mean rank	53.61	36.6	26.63	
N	22	36	19	
<b>Neutrophils count (x10<sup>9</sup>/L)</b>				<b>&lt; 0.05</b>
Median	3.61	2.5	2.37	
Mean rank	52.36	34.99	31.13	
N	22	36	19	
<b>Lymphocytes count (x10<sup>9</sup>/L)</b>				<b>&lt; 0.05</b>
Median	2.19	1.5	1.01	
Mean rank	54.43	37.04	24.84	
<b>Monocytes count (x10<sup>9</sup>/L)</b>				<b>&gt; 0.05</b>
Median	0.08	0.03	0.04	
Mean rank	45.66	36.22	36.55	
<b>Eosinophils count (x10<sup>9</sup>/L)</b>				<b>&gt; 0.05</b>
Median	0	0	0	
Mean rank	44	38.03	35.05	
<b>Basophils count (x10<sup>9</sup>/L)</b>				<b>&gt; 0.05</b>
Median	0	0	0	
Mean rank	38.73	39.11	39.11	

IFN- $\gamma$  levels appeared at undetectable median in HD patients and renal disease patients, while it appeared at 0.1 and 0.05 pg/ml median level in hepatitis and healthy group, respectively. However, the only significant difference revealed between HD patients and hepatitis patients ( $p < 0.005$ ). Whereas, IL-8 elevated levels have detected among all study groups, table 3, with significant differences among all study groups except between HD patients and renal disease patients.

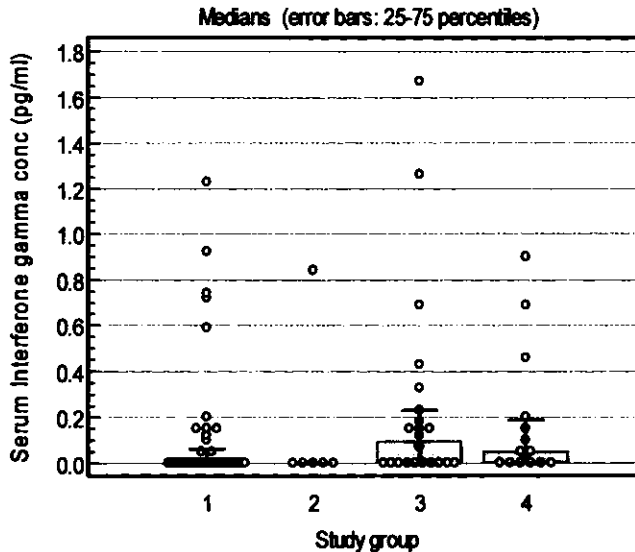


**Table 3 : The difference in median value of selected parameters between the 3 study groups.**

<b>Serum Interferon gamma conc (pg/ml)</b>					> 0.05	> 0.05		> 0.05
<b>Median</b>	0	0	0.1	0.05				
<b>Inter-quartile range</b>	(0 - 0.08)	(0 - 0.21)	(0 - 0.26)	(0 - 0.2)				
<b>Mean rank</b>	39.68	36.33	51.95	51.3				
<b>N</b>	45	6	22	15				
<b>Serum IL8 conc. (pg/ml)</b>						> 0.05		
<b>Median</b>	24.1	23.7	86.9	104.1				
<b>Inter-quartile range</b>	(23.6 - 27.8)	(19.6 - 25.4)	(19.6 - 539.4)	(90.2 - 295.2)				
<b>Mean rank</b>	33.46	23.58	54.59	71.2				
<b>N</b>	45	6	22	15				
<b>Serum CRP (mg/L)</b>								
<b>Median</b>	0	0	0	0				
<b>Inter-quartile range</b>	(0 - 12)	(0 - 0)	(0 - 0)	(0 - 0)				
<b>Mean rank</b>	98.73	70.4	70.68	63.5				
<b>N</b>	82	10	22	50				

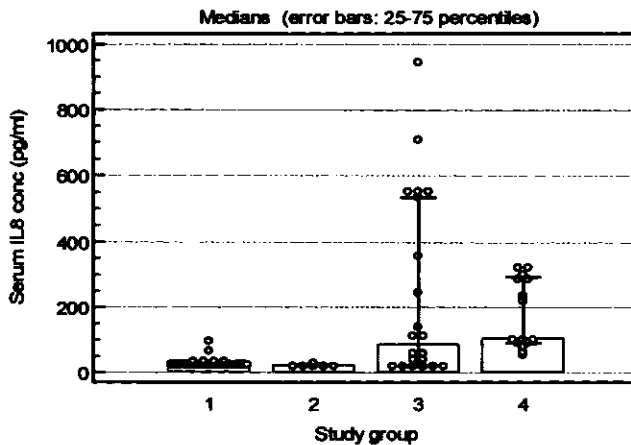
IFN- $\gamma$  is considered a major proinflammatory agent because it possesses various properties such as anti-tumoral cytotoxic type activity, stimulation of class II major histocompatibility complex (MHC) antigens on macrophages<sup>(19)</sup>, and production of superoxide and nitric oxide<sup>(20)</sup>. There are few studies involving a level of IFN- $\gamma$  in haemodialysis patients, to evaluated the immunological picture in these patients, and to assess the level of IFN- $\gamma$ , therefore, this study was considered the first study in Iraq. Previous studies have indicated IFN- $\gamma$  as regulatory factors of erythropoiesis by using recombinant human erythropoietin (EPO) in haemodialysis patients<sup>(21)</sup>.

Figure (1) shows the distribution of IFN-  $\gamma$  concentration in arrangement of (0-1.8) pg/ml among the four study groups; almost the cases are located between arrangement of (0-0.2) pg/ml of serum IFN-  $\gamma$ . Almost the readings results of all study groups focused in low concentration of INF-  $\gamma$ .

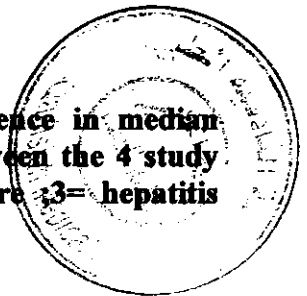


**Figure 1: Dot diagram with error bars showing the difference in median concentration (with its interquartile range) of serum IFN between the 4 study groups (1= Haemodialysis patients; 2=renal disease, not failure; 3= hepatitis patients ;4=healthy control ).**

From figure 2, one may see that The results presented in this study have showed the low levels of IL-8 concentration in two study groups (hemodialysis group and renal diseases group), figure (2) arrange lower than 50 pg/ml. the higher level were found in group 3(patients with hepatitis B&C) , healthy control (group 4) present moderate IL-8 level between groups(1,2) and group(3).



**Figure 2: Dot diagram with error bars showing the difference in median concentration (with its interquartile range) of serum IL8 between the 4 study groups(1= Haemodialysis patients; 2=renal disease, not failure ;3= hepatitis patients ;4=healthy control ).**



Stephen *et al.* , 2001, demonstrate significant increases in levels of IL-8 in HCV-infected patients compared to levels in uninfected patients, and patients who were biochemical nonresponders to IFN therapy had higher pretreatment levels of IL-8<sup>(22)</sup>. In other clinical studies, it has been demonstrated that chronic hepatitis C patients with high histologic activities have increased levels of IL-8 mRNA expression <sup>(23)</sup>.

In agreement with the present study, one previous study also find that serum IL-8 protein levels are elevated in HCV infected patients <sup>(24)</sup>. IL-8 is induced in multiple cell types by a variety of stimuli, including endotoxin, mitogens, and proinflammatory cytokines<sup>(25)</sup>. Many viruses and viral products can also induce the production of IL-8 that appears in the circulation during viral diseases<sup>(26,27,28,29)</sup>. Two receptors, CXCR1 and CXCR2, which are expressed by several cell types of hematopoietic and nonhematopoietic origin, mediate the biologic activity of IL-8 <sup>(30)</sup>. As for HBV, most studies have focused on vaccines, so we did not find study comparable with our results.

Data from recent study may emphasizes the principle of immune suppression of hemodialysis patients, other immune mediators such as IL-1, TNF are recommended to be evaluated in future studies.

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