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Evaluation of the Level of Interleukins 13 and 33 in the Serum of Patients with Renal Failure in Kirkuk City

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ABSTRACT
Background: A poor prognosis and a high death rate are associated with chronic progressive renal loss of parenchymal function.
Objective: This study aimed to ascertain the relationship between interleukin (IL)-13 and IL-33 and kidney failure and their function as inflammatory markers.
Patients and methods: Study participants ranged in age from 17 to 75 and were admitted to the Dialysis Unit of Kirkuk General Hospital. They included 45 clinically diagnosed patients (15 patients with *Candida albicans* infection and 30 without infection), and 45 healthy controls. Immunological tests of IL-33 and IL-13 were done using ELISA.
Results: The mean value of IL-13 was significantly higher among the cases group than in the control group (184.08 ± 60.79 vs 120.44 ± 22.67, P=0.000). Likewise, the mean level of IL-33 was significantly higher among renal failure patients than those in the renal failure-free group.
Conclusions: The present study showed increase in IL33 levels in serum of renal failure patients when compared with the control group. Whereas, the IL13 and IL-33 levels in the serum of renal failure patients with *Candida albicans* infection were higher than in renal failure patients without *Candida albicans*.
Keywords: Kidney failure, Chronic kidney disease, IL33, IL13, *Candida albicans*.

INTRODUCTION

The kidneys receive an abundant supply of blood, which they use to assess and modify the functioning of multiple organ systems. They are essential for filtering blood and are the principal organ in preserving the body's overall water balance and blood pressure [(1)]. Acute kidney injury (AKI) and chronic kidney disease (CKD), among other conditions, have a significant impact on global health and place a heavy strain on healthcare systems [(2)].

Additionally, older individuals with impaired renal function may be more vulnerable to developing functional limitations and disabilities [(3)]. Initially thought to be produced by leukocytes solely, interleukins are a class of cytokines that are now known to be produced by a wide range of different body cells; they are necessary for immune cell activation, differentiation, migration, adhesion, proliferation, and maturation. Furthermore, they possess both pro- and anti-inflammatory qualities [(4)].

An IL-1 family member called interleukin-33 is crucial for intestinal growth, and inflammation functions as an alarming cytokine in the gut [(5)]. IL-13 promotes fibrosis by increasing TGF-1 synthesis and activation or directly activating fibroblasts' synthetic and proliferative properties, smooth muscle cells, and epithelial cells [(6)]. As a result, this investigation aimed to determine the relationship between IL-33 and IL-13 in patients with chronic renal disease and their role as inflammatory markers.

MATERIALS AND METHODS

Sample collection:

From November 2021 to March 2022, we collected five milliliters of blood drawn from every patient and control individuals. From 45 patients with chronic renal disease admitted to Kirkuk General Hospital Dialysis Unit, and Forty-five healthy people formed the control group who were age- and sex-matched with the patients. All samples were centrifuged for 10 minutes at 3000 rpm. The isolated serum was collected and stored at -18 ˚C. We selected 15 blood samples from *C. albicans* patients and 30 blood samples randomly from other patients.

Immunological tests:

Human IL33 and IL13 ELISA kit (R & D Systems, USA):

The method of the test is a quantitative sandwich enzyme immunoassay. An anti-IL-33 or IL-13 antibodies were previously covered on a microplate. The immobilized antibody binds the pipette into the wells with standards, samples, and any IL-33 or IL-13 present. After removing any unbound substances, the wells were treated with an antibody coupled to biotin for IL-33 or IL-13. After washing, the wells were treated with horseradish peroxidase (HRP) and avidin. After wash, a substrate solution was added to the wells to remove any unbound avidin-enzyme reagents, and the color evolves proportionately to the amount of IL-33 or IL-13 bound in the initial step. It stops the color from developing and measures how intense a color is yellow. A microplate reader tuned to 450 nm was applied (HumaReader HS spectrophotometer, Medsource Ozone Biomedicals).

The immunological study:

A blood volume of five milliliters were drawn from the patients and the control groups for the blood samples. For 10 minutes, all of the samples were centrifuged at 3000 rpm. A serum that had been

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isolated was collected and frozen at -18 °C. Select 15 blood samples from *C. albicans* patients and 30 randomly from other patients. Forty-five most recent samples were taken from healthy controls.

Ethical approval:

The study was approved by the Ethics Board of AL-Iraqia University College of Medicine, and an informed written consent was taken from each participant or their parents in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

Statistical Package of Social Science (SPSS) version 26 was used to enter and analyze the data. For quantitative data, mean ± standard deviation, and range were used; frequency distribution tables, numbers, and percentages were employed for qualitative data. A logistic regression model and Roc curve were used to identify the optimal cut off value of immunological parameters as a predictive risk marker for kidney damage (renal failure) among study samples. A P-value of less than 0.05 was used as a significant criterion for all statistical tests done throughout the study.

RESULTS

The study showed significant differences between the study groups when comparing the immunological parameters of IL-13 and IL-33, as the mean value of IL-13 was found to be substantially greater among the cases group than that of the control group: (184.08 ± 60.79 vs. 120.44 ± 22.67) (P=0.000) . Likewise, the mean level of IL-33 was significantly higher among renal failure patients than in those of the renal failure-free group (170.21 ± 97.01 vs. 94.76 ± 13.08) (P=0.000) (Figure 1).

Comparing immunological characteristics across the cases group. Apart from control groups which have no positive *Candida albicans* culture and when we compared the patient's group with positive and negative fungal growth regarding the immunological parameters of IL-13 and IL-33, significant differences were detected, as the mean IL-13 value among patients with *Candida albicans* growth group was found to be substantially greater than that in the group with negative *Candida albicans* (220.9 ± 90.57 vs. 165.67 ± 24.39) (P= 0.003) with significant differences of 55.23. Similarly, the mean level of interleukin-33 was significantly higher among renal failure patients with positive *Candida* growth than those with negative growth (220.23 ± 144.14 vs. 145.19 ± 48.05) (P= 0.013) (Figure .(2

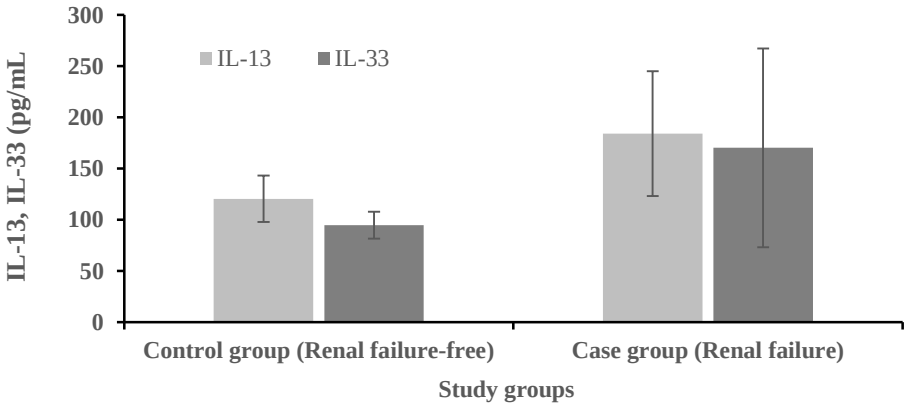

Figure (1): Mean comparison of the immunological parameter of IL-13 and IL-33 study groups (n = 90)

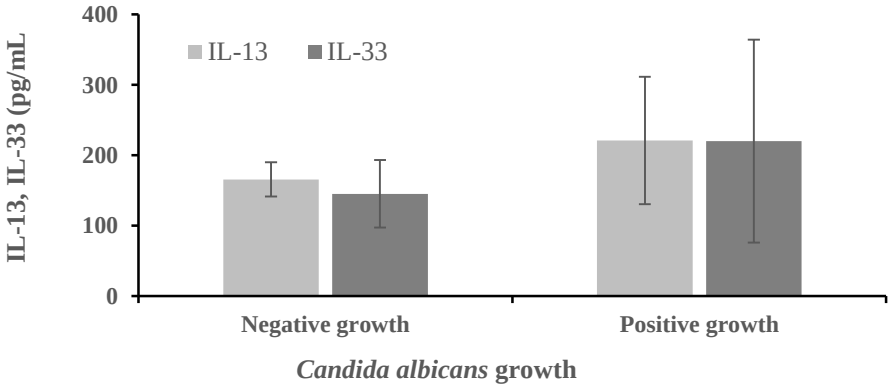

Figure (2): Mean comparison of the immunological parameter of IL-13 and IL-33 study groups (n = 90)

Immunological parameters as a predictive marker:
IL-13 as a predictive risk marker for kidney damage and renal failure:

Among a study sample, the optimal cut off value of IL-13 for detecting patients with a high risk of kidney damage and renal failure was 529.650 with a sensitivity of 97.8%, specificity of 88.9% and correctly predicted by the regression model of 91.1% with good area under the ROC curve (AUC) of 0.978 ± 0.012 (P= 0.000) (Table 1).

IL-33 as a predictive risk marker for kidney damage and renal failure:

Among a study sample, the optimal cut off value of IL-33 for detecting patients with a high risk of kidney damage and renal failure was 109.70450 with a sensitivity of 95.6%, specificity of 86.7% and correctly predicted by the regression model of 86.7 % with excellent space underneath the ROC curve (AUC) of 0.965 ± 0.018 (P= 0.000) (Table 1).

Table (1): Predictive value of interleukin-13 (IL-13) as a risk marker for kidney damage and renal failure (n=90)

Parameter	Sensitivity	Specificity	Accuracy	AUC	P-value
IL-13	97.8%	88.9%	91.1%	0.978± 0.012	0.000
IL-33	95.6%	86.7%	86.7%	0.965± 0.018	0.000

DISCUSSION

IL-33 is an IL-1 superfamily cytokine that stimulates T helper (Th) cells to produce type 2 cytokines [(7)]. The liver, kidneys, heart, brain, spleen, and lungs contain IL-33 [(8)]. Hospitalized patients frequently experience AKI as a complication. This disorder is related to an elevated long-term risk of adverse outcomes, including mortality, CVD, and CKD. Kidney function abruptly starts to decline, which is how it is identified as a disruption of fluid and electrolyte homeostasis over hours to days [(9)].

In the current study groups regarding the immunological parameters of interleukin-13 and interleukin-33 showed significant differences between renal failure patients and those of the renal failure-free group. This is in agreement with the results of **Oweis *et al*.** [(10)] that included 49 healthy controls and 65 hemodialysis (HD) patients. The average age of HD patients was 43.4 ± 21.3 years, whereas the average age of the control group was 31.5 ± 11.1 years. Patients receiving dialysis had mean blood levels of IL-13 of 8674.3 ± 4353.9 pg/ml and IL-33 of 42850.5 ± 11370.7 pg/mL.

In agreement with the current results , numerous inflammatory markers, including IL-1, IL-33, IL-18, IL-6, and TNF-, rise as chronic renal failure progresses [(11)]. Additionally, it has been reported that in individuals with chronic renal failure, IL-33 and ST2 rose as the CKD stage progressed [(12)]. In current study groups regarding the immunological parameters of IL-13 and IL-33 showed significant differences between renal failure patients and those of the renal failure-free group. This is in disagreement with previous studies reported by **Bao *et al.*** [(13)] who discovered that Il-13 and IL-33 were comparable in both groups of patients and control, while ST2 levels were more significant in patients with CKD than in healthy controls? They reported that as CKD stages advance, IL-33 and ST2 levels increase. Those with elevated sST2 expression in a cohort study of critically ill patients had the worst prognosis [(14)]. Additionally, ST2 and IL-33 levels have been found to rise as the stages of CKD progress [(12)]. Indeed, those with elevated sST2 expression fared the worst in a cohort study of critically ill patients.

The present study can state that chronic renal failure is connected with a rise in IL-33 levels. Additional research will clarify how IL-33 contributes to chronic kidney injury and its earliest phases. Moreover, this is the first study correlating this marker (Interleukin 13) and renal failure. Therefore, our findings have significant clinical ramifications for those with compromised immunity and opportunistic inflammation.

CONCLUSION

Finally, the present study can state that chronic renal failure is connected with a rise in IL-33 serum levels. Additional research will shed light on IL-33's function in chronic kidney failure and the early stages of renal damage. In this investigation, the blood IL-13 and IL-33 levels in kidney failure patients were increased with high significant relation, which is helpful indicator of disease activity with a high risk of kidney damage and renal failure with a cut off that was 109.70450 with a sensitivity of 95.6%, specificity of 86.7% and accuracy of 91%. The present study concluded increase in IL33 and levels of serum of renal failure patients when compared with the control group. Whereas, the IL13 and Il 33 levels in the serum of renal failure patients with Candida albicans infection were evaluated than in renal failure patients without Candida albicans.

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Author contribution: Authors contributed equally to the study.

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