Association of Gene Polymorphisms, Serum Level of Th17 Cytokines and HCMV Infection in Renal Failure Patients

A Thesis Submitted to
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1.1   Introduction

Renal failure a type of a common kidney disease in world wide , that occurs by reducing kidney function , and representing progressive disease . Estimation of the reduction kidney function happened via glomerular filtration rate (GFR) , when was less than 60 mL / min (Ricci *et al*. , 2012).

Kidney plays an important role in regulation of blood fluid and composition , filtration of urine by eliminated the metabolic wastes , secretion of end metabolism products and maintenance of the acid or balance in body via regulate fluids and electrolytes (Aitken *et al* . , 2014) .

Kidney disease (KD) was related with age because kidney function is decline during the time and that lead to accelerate in diabetes, obesity, hypertension and primary kidney disorder (Pscheidt *et al* . , 2015).

Human cytomegalovirus (HCMV) is a DNA virus that belong to herpesvirdia, prevalence rate of virus was widely in population because this virus is endemic in many countries. Prevalence rate of HCMV disease in all world is based on immune status and patient's age. In immunosuppression patients include renal failure, cancer, and others, this virus cause opportunistic infection, while in immunocompetent patients is rare. HCMV is presented in body fluids, and transmitted by genital tract, breast milk, blood transfusions, and organ transplant (Jassima *et al*., 2014). More incidence of viral infections in renal failure patients because this patients suffer from reduce of immune response , with kidney transplant, and blood transfusion were contribute of HCMV transmission and cause infection. This infection may be primary, or more commonly via reactivation by latent HCMV or reinfection by exogenous virus (Vilibic-Cavlek *et al*., 2015).

In addition to, some factors such as contamination dialysis apparatus and number times of blood transfusion during hemodialysis methods. These lead to increase risk of factors in receiving CMV in these patients. Prevalence rate of CMV infection among renal failure patients about is 66 - 84% (Khalafkhany *et al*., 2016).

Immune response occurs by innate immunity Natural Killer "NK" cell and adaptive immunity antibody, $CD4^+$ and $CD8^+$ T-cells, was played a main role in protection against HCMV infection and Coordinated effect of these responses which lead to reducing infection by controlling of viral replication. After infection with viruses , activation and differentiation of $CD8^+$ T cells occurs from naive cells to effector T cells , which play a role in controlling virus replication via cytolysis and secretion some cytokines specific to virus , and memory cells that support and enhance immunity after other infection with same pathogen (Litjens *et al*.,2017) . Activation of macrophages , production of cytolytic molecules , production of cytokines , also help to B-cell and CD8+ T-cells , these are represent several effector functions , $CD4^+$ T-lymphocytes had ability to produce these functions to resist virus infection. $CD4^+$ T cells classified to two type according to different cytokine producing: T–helper include (Th1, Th2 and Th17) and regulatory T (Treg) cells (Ciccocioppo *et al*., 2015).

T–helper 17 cells Stimulate inflammation via secretion IL-17A (IL17), IL-17F, IL-21 and IL-22 and causing organ injury. Also, act on enhancing produce pro-inflammatory cytokines by resident cells , that lead to increase of penetration of neutrophils to affect organ and reduce inflammation and damage in this organ (Zhao *et al* . , 2018) .

Cytokines are proteins, low molecular weigh, and producing from different types of immune cells to contribute in response against infection and organ damage. Cytokine production may be effected by cytokine gene Polymorphisms by effect of this gene on transcription. Single nucleotide polymorphisms (SNPs), act on induce susceptibility or resistance to any infection, and may be use to diagnose some factors that contribute to cancers or inflammatory disease SNP occurs in regulatory regions for cytokine gene (Medrano and de Oliveira , 2014) .

1.2   **Aims of Study**

1- Detection of cytomegalovirus DNA by polymerase chain reaction (Real Time PCR) in renal failure patients.

2- Investigate about the level IL17A, IL17F, IL21 and IL22 in serum renal failure patients.

3- Study the role of cytokine gene SNP of IL17A, IL17F, IL21 and IL22 in renal failure patients by detection a single nucleotide polymorphism (SNP) genotyping for all these cytokines.

2.1 Renal Failure

2.1.1 Kidneys: Structure and Functions

Kidneys are two important organs that found on the side of Vertebral column, bean shape, color is reddish Brown and covered by renal capsule (Agur and Dalley , 2009). This organ play main role in the conservation of common body function and formation of urine was the main function by completely filtration, reabsorption and secretion mechanism. Additionally, kidneys regulate electrolyte, fluid, and acidity balance in the body and produce a stable environment for the metabolic routes of tissues and cells. (Tzanakaki *etal.,2014*). kidney also secreted 3 important hormones : Erythropoietin : stimulates the creation of Red Blood Cells RBC , Renin: regulates blood pressure and Calcitriol : active form of vitamin D that regulate blood calcium levels.

2.1.2 Renal Failure or Kidney Disease

Renal failure is acommon disease , usually is defined as the decrease of kidney function , glomerular filtration rate (GFR) which used to estimate the kidney function reduction which is less than 60 mL / min , levels of this disease now as 9th in cause of death , According to the Global Burden of Disease (Akpan and Ekrikpo ,2015) .

In Worldwide, there is an obvious increase in morbidity and mortality with end-stage renal disease (ESRD), as a result to increases morbidity of non-infectious diseases. In Iraq, database around the prevalence of ESRD was in lack and the known about the problem was less too. Also, the capability of the health system to manage the disease non efficient (Alaugili and Alami , 2015) .

Summary

The study included 200 Iraqi Patients with renal failure at age ranging 12-75years from Ibn Sina Center for Dialysis and kidney disease in Baqubah Teaching Hospital and 50 healthy controls at age ranging 12 – 49 during the period from February until November 2018.

The Patients in this study included (126 males and 74 females) and healthy controls (24 males and 26 females) . First step included diagnosis of HCMV in studied groups and second step represents estimation of immunological role of Th-17 cytokines, while final step includes detection of several Single Nucleotide Polymorphisms (SNP) associating with these cytokines.

The results showed the prevalence of HCMV DNA in renal failure Patients was 24%. As well estimation of the interleukins level in serum by using (ELSA) test shown that Patients had a high significant compared with controls in ($p<0.001$).

In addition to the level of interleukins was none effected by: HCMV infection, age, and sex. The result of IL-17A (rs2275913) polymorphisms Gene showed that a correlation between this SNP and renal failure Patients, the results showed: GA, AA and GG genotypes were detected in 26 %, 16 % and 58% Patients, and in 13 %, 27 % and 60 % controls, respectively. While According to IL-17F (rs763780) polymorphisms Gene, results showed: TC, CC and TT genotypes were detected in 7 %, 6 % and 87 % Patients, and 3 %, 7% and 90% controls, respectively, also TC genotype is considered as etiological factor.

Also, IL-21 (rs2221903) polymorphisms Gene showed correlation between this SNP and renal failure Patients, those results showed: - GA, GG

Kidney disease (KD) is related with age because the function was decline during the time and that lead to accelerated in diabetes, obesity , hypertension, and primary kidney disorder (Gansevoort *etal.* ,2013). There are two type of kidney infection:

1-   Acute Kidney Disease (AKD) or Acute Renal Failure (ARF)

Acute Kidney Disease AKD was a type of renal failure characterized by a fast decline of kidney function. Accumulation of fluid , electrolytes and waste products , was clinical diagnosis of AKD another reason , had less observable effects , including immunity in reduced and disorder function of other organ such as liver , brain , heart, and lung non-renal organs (Singbartl and Joannidis , 2015) .

The rapid lossed of kidneys function was produced from Acute Kidney injury (AKI), that lead to keeping of waste products, electrolyte disruption , and changes in volume status . Acute kidney disease, this term had exchanged acute renal failure, with simpler changes in renal work without overt failure can cause in important clinical results and increased morbidity and mortality.

Biomarkers can be used to detect the changes in kidney function , serum creatinine (SCr) is more commonly , and this test is an defective biomarker for diagnosed AKI , by increased in SCr often lasts 48–72 hours behind the onset of injury. Additionally, SCr was a label state in critically ill patients , leading to incorrect estimates of glomerular filtration rates (GFRs).''(Awdishu *etal* . , 2016) .

The risk factors for AKI infection include: Age, nephrotoxic exposures, comorbid diseases, major surgery, proteinuria, sepsis, volume status and fluid resuscitation. Elder age rises the risk of AKI (Hsu, 2008). AKI could be classified into three types of groups according to Causes: