Candin Test in Evaluation of Cellular Immunity in Chronic Kidney Disease Patients - A Cross Sectional Observational Study

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ABSTRACT

Introduction: Chronic kidney disease (CKD) patients are more susceptible to infection due to impaired immune competency. Age, nutritional deficiencies, uremic toxins, dialysis, metabolism of parathyroid hormones and immunosuppressive medications contribute to immune dysregulation. Aims of the present study were to find out the use of Candin Test to early reorganization of immunocompromised state of cellular immunity in patients of CKD and to find out correlation between Candin Test results and various factors altering immune system in CKD patients.

Material and methods: The Cross-sectional observational study was conducted on eighty adult patients qualifying the diagnostic criteria as per KDIGO guideline. Another age and sex matched eighty healthy volunteers were selected as control group. Patients having diabetes, HIV positive, malignancy, pregnancy, who are on steroids or any other immunosuppressive therapy, and those belonging to extremes of ages i.e.<18 years and >65 years were not included in the study. A detailed history, clinical examination and relevant investigations (Serum urea, creatinine, FBS, HIV, iPTH and USG abdomen) were done in each subject. Each subject also underwent Candin Test to assess the level of cellular immunity.

Result: Out of 80 cases, 35% patients showed positive induration while control group (n=80) demonstrated 58.8% indurations that revealed significantly more induration positive in controls (p=0.0024). In stage 3 CKD patients, 55.5% cases showed positive induration, however positive induration decreases 52% and 24% in stage 4 and stage 5 respectively. Induration was significantly more positive in stage 3 and stage 4 in comparison to stage 5 (p=0.007). Longer duration of CKD showed lesser number of positive induration response but difference was statistically non-significant (p=0.0521). There was significantly more induration response in no hemodialysis group (p=0.032) in comparison to hemodialysis group. No significant difference was observed between induration and age. Mean eGFR of the patients with positive induration was 20.14±13.083 and those without induration was 12.87±7.968 with p=0.003, which is significant i.e. cases with positive induration have higher eGFR values. Mean Serum iPTH is 215.50±119.279 in patients with positive induration and 312.88±286.601 in patients with no induration which is not significant (p=0.091).

Conclusion: Our study emphasises that Candin Test may be useful to predict anergy in patients of CKD. As the CKD patients approaches towards ESRD, cellular immunity also decreases. It has also been observed that Candin Test response was diminished in patients on maintenance HD, which explains that as the frequency of HD increases patient's immunity decreases.

Keywords: Anergy, Candin Test, Cellular Immunity, Chronic Kidney Disease.

INTRODUCTION

The global burden of chronic kidney disease (CKD) is considerable and has risen dramatically over the past 20 years.[1] GBD 2016 report documented that CKD had rapidly moved up the ranks of causes of global deaths and is currently positioned at 11th on the list.[2] CKD patients are more susceptible to infection due to impaired immune competency and frequent exposure to infectious agents due to frequent dialysis session. After cardiovascular disease, infectious diseases are the second most common causes of morbidity and mortality in patients with CKD, contributing to 30–36% of deaths among patients on dialysis.[3,4] Age, nutritional deficiencies, uremic toxins, dialysis, metabolism of parathyroid hormones and immunosuppressive medications contribute to immune dysregulation. CKD affects both major immune systems include innate and adaptive responses. The innate system is a rapid, effective, and universal form of defense against infections, driven by polymorphs, macrophages, and dendritic antigen-presenting cells (APC). The adaptive immune system is antigen-specific, requires recognition of processed antigen, and is driven through activated T and B lymphocytes. Cellular or delayed-type hypersensitivity (DTH) can be assessed by intracutaneous testing with bacterial, viral and fungal antigens to which most healthy persons are sensitized. A positive skin test denotes prior antigenic exposure, T-cell competency and an intact inflammatory response.[5] The reaction usually peaks 48 hours after antigen is introduced into the skin and is manifest as indurations at the test site. Candida albicans skin test antigen for cellular hypersensitivity (Candin Test) are useful in the assessment of diminished cellular immunity in persons infected with human immunodeficiency virus, moreover responses to DTH antigen have also reported to have prognostic values in patients with cancer but it is for the first time Candin antigen has been used in CKD patients to assess DTH response. There is no published study of Candin

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Test in CKD patients. The current study focuses on current clinical immunity among patients with CKD. Aims of the present study were to find out the use of Candin Test to early reorganization of immunocompromised state of cellular immunity in patients of chronic kidney disease and to find out correlation between Candin Test results and various factors altering immune system in CKD patients.

MATERIAL AND METHODS

The Cross-sectional observational study was conducted on eighty adult patients qualifying the diagnostic criteria for CKD as per KDOQI guidelines.[6] Another age and sex matched eighty healthy volunteers were selected as non CKD group attendant outpatient and inpatient department of the Hospital. Patients having diabetes, HIVpositive /AIDS patients, malignancy, pregnancy, who are on steroids or any other immunosuppressive therapy, and those belonging to extremes of ages i.e.<18 years and >65 years were not included in the study.

A detailed history, clinical examination and relevant investigations were done in each subject to confirm the disease, assess the disease progression and categorize the patients in different stages of CKD. Each subject also underwent Candin Test to assess the level of cellular immunity. History included duration of symptom, drug intake, number of dialysis undertaken and any concurrent or chronic illness. Relevant investigations were also done. Serum urea, serum creatinine, serum intact PTH and ultrasonography of the abdomen(for renal size and echotexture)were done in the hospital for confirming CKD. Fasting blood glucose and Post-prandial blood glucose were measured to rule out diabetes. HllV test was also done to rule out any such concurrent illness which leads to immunocompromised state. Candin Test was done all the cases and controls. Candin was administered intradermally on the volar surface of the forearm with a test dose of 0.1 ml. The skin was cleansed with 70% alcohol before applying the test. Induration response was read in all the individuals after a period of 48 hours, Mean induration in control population was 5.3 mm, so any induration of ≥5 mm was considered positive among cases.

Candin Test

Intradermal candida albicans Skin Test Antigen for cellular hypersensitivity is a clear, colourless, sterile solution with a pH of 8.0 - 8.5. It is made from the culture filtrate and cells of two strains of Candida albicans. The fungi are propagated in a chemically defined medium consisting of inorganic salts, biotin and sucrose. Lyophilized source material is extracted with a solution of 0.25% NaCl, 0.125% NaHCO3 and 50% v/v glycerol. The concentrated extract is diluted with a solution of 0.5% NaCl, 0.25% NaHCO3, 0.03% Albumin (Human), 8 ppm polysorbate 80 and 0.4% phenol.

The skin test strength of Candin has been determined from doseresponse studies in healthy adults. The product is intended to elicit an induration response ≥ 5 mm in immunologically competent persons with cellular hypersensitivity to the antigen. Measurements should be made across two diameters. The mean of the longest and midpoint orthogonal diameters

of the indurated area should be reported as the DTH response. For example, a reaction that is 10 mm (longest diameter) by 8 mm (midpoint orthogonal diameter) has a sum of 18 mm and a mean of 9 mm. The DTH response is therefore 9 mm.

eGFR estimation

Creatinine (by Modified version of Brod et al. method) is treated with picric acid in alkaline medium, a red colour develops which is measured calorimetrically. The reaction is not specific but at least over 85 percent colour is due to creatinine. A number of formulae have been devised to estimate GFR or Creatinine clearance (Ccr) values on the basis of serum creatinine levels.Estimated creatinine clearance rate(eCc)using Cockcroft-Gault formula.A commonly used surrogate marker for estimation of creatinine clearance is the Cockcroft-Gault formula,which in turn estimates GFR in ml/min:It is named after the scientists who first published the formula,and it employs serum creatinine measurements and a patient's weight to predict the creatinine clearance

The most recently advocated formula for calculating the GFR is the one that was developed by the Modification of Diet in Renal Disease Study Group.For creatinine in mg/dl: EGFR=186 x Serumn Creatinine-1.154x Age -0.203 x{1.212if Black} x {0.742 if Female}

STATISTICAL ANALYSIS

Collected data were tabulated, and quantitative parameters were expressed as mean and standard deviation, whereas qualitative variables were presented as frequency and percentages. Two-tailed independent unpaired t-test and Chi-square test were used to establish association. Statistical significance was defined at a p value of 0.05.

RESULT

Out of 160 subjects, 80 cases (male;female, 47;33) and 80 controls (male;female, 44;36) were enrolled in the cross sectional observational study. Cases were non-diabetic CKD stage 3 to stage 5 while controls were non-diabetic non-CKD healthy volunteers. Cases and controls both were age and sex matched. Mean age of the cases was 42.3±11.7 years while for controls it was 38.2±10.1 years.

All the enrolled subjects were injected intradermal Candin antigen and evaluated for induration after 48 hours. Among cases 28 out of 80 i.e. 35% show positive induraion (>5 mm) for candin test, while in control population, 47 out of 80 i.e. 58.8% show mean induration of 5.3±1.7 mm. The observations revealed significantly more induration positive in controls (p=0.0024) (Table 1).

Figure 1 shows distribution of cases according to the stage of CKD, 63% were in stage 5, 26% were in stage 4 and least

Groups	Induration n(%)	No induration n(%)	P value
Case	28 (35.0)	52 (65.0)	0.0024*
Controls	47 (58.8)	33 (41.2)	
Total	75 (47.5)	85 (52.5)	
Table-1: Comparison between cases and controls with induration (>5 mm)			

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Variables	Induration (>5 mm) n(%)	No induration (<5 mm) n(%)	p value
Stage of CKD			
Stage 3	5 [55.5]	4 [44.5]	
Stage 4	11 [52.3]	10 [47.7]	**p=0.007***
Stage 5	12 [24.0]	38 [76.0]	
Duration of CKD			
<6 months	16 (47)	18 (53)	p=0.052
>6 months	12 (26)	34 (74)	
Frequency of hemodialysis/week			
0	11(55)	9 (45)	
1-2	9 (34.6)	17 (65.4)	**p=0.032***
>2	8 (23.4)	26 (76.6)	
Age			
<40 years	14 (46.7)	16 (53.3)	p=0.091
≥40 years	14 (28)	36 (72)	
*p=<0.05 demonstrate statistically significant at CI 95%			
Table-2: Candin Test induration (>5 mm) in CKD patients (n=80).			

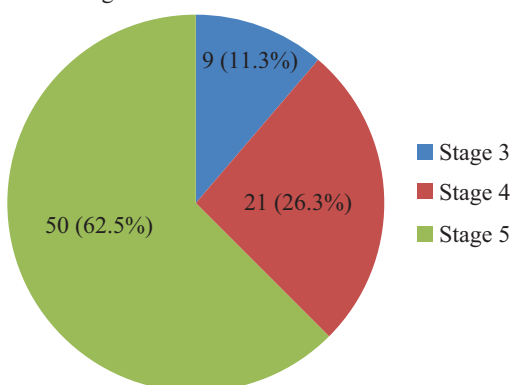| Variables	Induration (>5 mm) (n=28)	No induration (<5 mm) n(n=52)	p value
eGFR (mean±STD)	20.14±13.083	12.87±7.968	p=0.003*
Serum iPTH (mean±STD)	215.50±119.279	312.88±286.601	p=0.091
(*p=<0.05 demonstrate statistically significant at CI 95%)			
Table-3: eGFR and serum iPTH in CKD patients.			

CKD stage wise cases distribution

Figure-1: CKD Stage wise cases distribution

number of cases (11%) were in stage 3. No patient belonged to stage 1 and 2. Out of 80 cases, 46 cases i.e. almost 58% were diagnosed as CKD for more than a period of 6 months and 36 cases i.e. 42% were newly diagnosed as CKD for a period of less than 6 months.

In stage 3 CKD patients, 55.5% cases showed positive induration (>5 mm), however as the stage progressed percentage of the patients with positive induration decreases i.e. 52% and 24% in stage 4 and stage 5 respectively. Induration was significantly more positive in stage 3 and stage 4 in comparison to stage 5 (p=0.007) (Table 2). Furthermore CKD patients who were having a longer duration of disease showed lesser number of positive induration response but difference was statistically non-significant (p=0.0521). There was significantly more induration response in no hemodialysis group (p=0.032) in comparison to hemodialysis group. No significant difference was observed between induration and age (Table 2).

Mean eGFR of the patients with positive induration (>5 mm) was 20.14±13.083 and those without induration (<5 mm) was 12.87±7.968 with p=0.003, which is significant i.e. cases with positive induration have higher eGFR values. Mean Serum iPTH is 215.50±119.279 in patients with positive induration and 312.88±286.601 in patients with no induration and p=0.091 which is not significant (Table 3).

DISCUSSION

Chronic renal failure is associated with a state of immune dysfunction characterized by immune depression that contributes to the high prevalence of infections among these patients. The major causes of death in CKD patients are cardiovascular and infections, together accounting for 70% of all CKD deaths.[7,8] Although it is well established that a dysfunction of the immune system is induced by the uremic milieu, this ominous disturbance has not been systematically studied as a potential contributing cause of premature deaths resulting from CVD and infections in ESRD. It should be noted that the immune dysfunction in uremia is associated with alterations in the two major branches of the immune system, innate and adaptive immunity.

There is a wealth of evidence that disorders of both innate and adaptive immune systems contribute to an increased rate of infections in the course of ESRD. Functional abnormalities of monocytes, neutrophils and dendritic cells are directly linked with infection risk in this patient population.[9-13] Impaired maturation of the lymphocytes, seen in dialysis subjects, also lead to disabled immune response and infection susceptibility.[9]

High failure rates for vaccinations against hepatitis B virus, influenza virus, Clostridium tetani, or corynobacterium diphtheriae are also thought to be caused by alterations of T lymphocyte functions.[14] Several studies have been done

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in the past to evaluate the level of immunity in patients of chronic kidney disease and its contributory factors. Anergy skin testing assesses the responses to skin-test antigens to which cell-mediated, DTH response is expected. Anergy or DTH tests placed by using Mantoux method(skin testing for PPD, candida and mumps antigen) of intradermal injection have conventionally been classified as positive if an induration measuring greater than or equal to 5 mm is observed at the injection site within 48-72 hours. Persons who have positive skin tests are considered to have relatively intact cell-mediated immunity. Persons who do not mount a DTH response are considered to be anergic and to be at elevated risk for complications of deficient cell-mediated immunity.

In the present study, we have used Candida antigen (Candin Test) to assess the level of cellular immunity on the principles of DTH reactions. A total of 80 cases (47 males and 33 females) and 80 controls (44 males and 36 females) were enrolled in this cross-sectional observational study. Cases were non-diabetic CKD belonging to stage 3 to stage 5 patients while controls were non-diabetic non CKD healthy volunteers. Mean age and standard deviation of cases was 42.3±11.7 years while for controls it was 38.2±10.1 years and the age of the cases was significantly higher than the controls (P=.021). Firstly, Candin Test was done on healthy volunteers. 47 i.e.58.8% individuals out of a total of 80 controls show induration response after 48 hours with a mean induration of 5.3 mm. As some persons with normal cellular immunity are not hypersensitive to Candida, a response rate less than 100% to the antigen is to be expected in normal individuals. Therefore, the concurrent use of other licensed DTH skin test antigens is recommended, however this is beyond the scope of this study. Since, mean induration in control population was 5.3 mm, any induration of size >5 mm in cases was considered to be positive response.

Out of 80 cases of CKD, 63% were in stage 5,26% were in stage 4 and least No. of cases i.e.11% were in stage 3. However, there were no patients of stage 1 and 2.46 subjects i.e. almost 58% were diagnosed as CKD for more than a period of 6 months, and 34 subjects i.e.42% were newly diagnosed as CKD for a period of less than 6 months.

On applying Candin Test to the cases, 28 out of 80 i.e.35% show positive induration (≥5mm), while in control 58.8% were positive ;P=.002, so induration was significantly more positive in control population who were immunocompetent. Mean eGFR of the patients with positive induration (≥5mm) was 20.14±13 and those without induration (<5mm) was 12.8±8 with P=0.003, which was significant i.e. cases with positive induration have higher eGFR values.

In the present study on applying Candin Test to CKD patients, induration response (≥5mm) varied according to the stage of CKD patient. In stage 3 CKD patients, more than 50% cases showed positive induration (≥5mm), however as the stage progressed, percentage of the patients with positive induration response decreased i.e.52% and 24% in stage 4 and 5 respectively.

Induration was significantly more positive in stage 3 and 4 than in stage 5 (P=0.007) immunity of CKD patients decreases as ESRD approaches. This result is in conformity with several studies done by Brinkoetter et al.[15] and Meier et al.[16], which showed defective cooperation between T cells and APCs(especially monocytes) leading to subnormal proliferation and interferon-y and interleukin(IL)-2 production and thus reduced cellular immunity and DTH response. The numbers of circulating T-lymphocytes are reduced in HD and the reduction of T helper cells is more prominent than T suppressor cells. In studies done by Litjens et al.[17] and Yoon et al.[18] it was found that hemodialysis reduces the cellular immunity by functional impairment of T cells. In our study induration response was significantly (P=.03) more positive in no hemodialysis group which suggests that HD reduces immunity in conformity with other studies.

In our study on CKD patients who were having a longer duration of disease showed lesser number of positive induration response but statistically no significant difference was found (P=0.052),i.e. duration of disease has no direct correlation with immunity dysfunction.

According to the study conducted in 49 Finnish uremic CKD patients by Huttunen et al[19], age correlated inversely with the lymphocyte proliferation response to mitogens, however in our study no significant difference was found between younger(<40 years)and older(>40 years) group of CKD patients. In the study published by Chow et al[20], odds of non-response to hepatitis B vaccination is high for increasing age among dialysis.

In the present study mean Serum iPTH is 215.5 in patients with positive induration response and 312.8 in patients with no induration and which is not significant showing no correlation between induration response and level of Serum iPTH but Ori et al.[21] studied that as a result of excess amounts and action of PTH in CKD patients an elevated level of cytosolic calcium is seen which is associated with impaired cellular responses which is against our results.

There was some limitations of the study includes small sample size however sample was sufficient to demonstrate significant results. In the study only CKD patients of stages 3-5 were taken and Skin test was done with only a single antigen, use of additional antigens could increase the predictive value of DTH reaction. There was no follow-up DTH test done on patients.

CONCLUSION

Our study emphasises that Candin Test may be useful to predict anergy in patients of CKD. As the eGFR value decreases and CKD patients approaches towards ESRD, cellular immunity also decreases and thus, DTH response is diminished. It has also been observed that Candin Test response was diminished in patients on maintenance HD, which explains that as the frequency of HD increases patient's immunity decreases. Candin Test is easy to administer, its affordable and a very simple way to determine immune compromised state in CKD patients, and thus, can separate the anergic group of CKD patients from non-anergic group.

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Thus, we can determine the prognosis, predict susceptibility to infections, vaccinaton failures and derive therapeutic benefit accordingly.

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