

Evaluation of Delayed-Type Hyper Sensitivity Skin Test In CKD Patients: Significance of Candin Test

Arvind G¹, Upma N^{2*} and Nikesh M³

- 1 Division of Nephrology, Moti Lal Nehru Medical College, Allahabad, Uttar Pradesh, India
- 2 Tejas Microdiagnostics, Allahabad, Uttar Pradesh, India
- 3 Department of Medicine, Moti Lal Nehru Medical College, Allahabad, Uttar Pradesh, India

Abstract

Background: The high morbidity and mortality of patients with end stage renal disease have led to the development of a variety of guidelines for improving the care of patients on dialysis, and more recently the care of patients with early stages of chronic kidney disease.

Methods & Findings: A prospective observational study was done on 240 non-diabetic chronic kidney disease patients and on 240 controls to assess the delayed hypersensitivity response by using Candin test.

During Oct 2015 to July 2017 we enrolled 480 subjects and all were injected Candin antigen intradermally and evaluated for induration after 48 hours. Among these cases 35% patients showed positive induration while control group revealed 58.8% indurations. Induration was significantly more positive in stage 3 and 4 in comparison to stage 5. Cases with positive induration have higher eGFR value. Induration response was significantly more positive in the group which has not undergone for haemodialysis.

Conclusion: The study concludes that by using Candin test we can single out in advance those patients who are in the state of deteriorating cellular immunity in order to provide additional therapy to improve quality of their lives and also vaccinate such patients with augmented regimens for providing extra protective cover for reducing the morbidity from various infections. During this study period no CKD patient of stage 1 and 2 has been enrolled but if the Candin skin test had been done on such patients, encouraging results would have been got by protecting them with several infections.

Keywords: Candin; Delayed hypersensitivity testing; Cellular immunity; CKD; Heamodialysis

*Corresponding author: Upma N

✉ upmanarain@gmail.com

Tejas Microdiagnostics 62, Jawahar Lal Nehru Road, Tagore Town, Allahabad, Uttar Pradesh, India – 211002.

Tel: +919415253337

Citation: Arvind G, Upma N, Nikesh M (2018) Evaluation of Delayed-Type Hyper Sensitivity Skin Test In CKD Patients: Significance of Candin Test. Arch Clin Microbiol. Vol.9 No.2:81

Received: April 10, 2018; Accepted: April 28, 2018; Published: April 30, 2018

Introduction

Chronic kidney disease (CKD) patients are at increased risk of incidence and severity of infections because of their impaired immunocompetency and greater exposure to microbes due to frequent contacts with medical care facilities for diagnosis or treatment, including repeated dialysis sessions [1,2]. However, CKD patients show remarkable deterioration of immunologic functions, which decreases development of protective antibodies in response to vaccination compared to healthy subjects [3].

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 [4,5]. The reaction usually peaks 48 hours after antigen is introduced into the skin and is manifest as indurations at the test site.

The aim of this study is the use of Candin tests (a kind of DTH test) for early reorganization of immunocompromised state in CKD patients for providing additional supportive therapies and augmented regimens of vaccines to reduce the morbidity for improving their quality of life.

Methods

A prospective observational study was done on adult patients qualifying the diagnostic criteria for CKD as per kidney disease outcomes quality initiative (KDOQI) guidelines [6] and on non CKD group attendant outpatient and inpatient department of SRN Hospital during June 2014 to July 2016. Demographic data was recorded and a detailed history was taken from each patient as previous episodes of acute kidney injury, symptoms of uremia, duration of symptoms, number of dialysis undertaken and any concurrent or chronic illness. Clinical examination including blood pressure measurement, pallor, anasarca, chest crepitations, and pericardial rub were assessed. Baseline investigations were done for enrolled patients to confirm the disease, to assess the disease progression, to categorize the patients in different stages of CKD. USG abdomen was done for renal size, echogenicity and corticomedullary differentiation. Each subject also underwent for Candin test to assess the level of cellular immunity.

Candin Test

Candida albicans Skin Test Antigen is a clear, colour-less, 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. A ready-to-use standardized *Candida albicans* skin test product (Candin) became first available in 1995 [7].

The antigen should be administered intradermally. The skin-test strength of Candin has been determined from dose-response 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.

Statistical analysis was performed using chi square test, student t test and contingency coefficient. Data were expressed as mean \pm standard deviation. Statistical significance was defined at a p value of 0.05.

Results

Out of 480 subjects, 240 cases and 240 controls were enrolled for this study. Cases were non-diabetic CKD from stage 3 to stage 5 while controls were non-diabetic non-CKD volunteers. Mean age of the cases was 42.3 ± 11.7 years while for controls it was 38.2 ± 10.1 years. Among the cases 41% were females and 47% were males while under control group 45% were females and 55% were males. All the enrolled subjects were injected intradermal Candin antigen and evaluated for induration after 48

hours. **Figures 1-3** illustrates the induration response in stage 3 and stage 5 and **Table 1** depicts the results of induration among cases and control.

Among cases 84 (35%) patients showed positive induration (>5 mm) while control group revealed induration in 141 (58.8%) individuals for Candin test. **Table 2** illustrates stage wise distribution of cases showing response after Candin Test.

During this study period no CKD patient of stage 1 and 2 has been enrolled. In stage 3, 55.5% cases showed positive induration (>5 mm) and as the stage progressed number of percentage of the patients with positive induration decreased i.e. 52.3% and 24.0% in stage 4 and 5 respectively with significant p value of .007 (Chi square test). **Table 3** depicts association of eGFR and induration.



Figure 1 Induration in CKD stage 3.



Figure 2 Induration in CKD stage 4.



Figure 3 Induration in CKD stage 5.

Table 1 Results of induration among cases and control.

Groups	Induration (%)	No Induration (%)	Total
Case	84 (35.0)	156 (65.0)	240
Controls	141 (58.8)	99 (41.2)	240
Total	225 (47.5)	255 (52.5)	480

Table 2 Stage wise distribution of cases showing response after Candin Test.

Stage of CKD	Induration (%)	No Induration (%)	Total
3	15 (55.5)	12 (44.5)	27
4	33 (52.3)	30 (47.7)	63
5	36 (24.0)	114 (76.0)	150
Total	84 (35.0)	156 (65.0)	240

Table 3 Association of eGFR and Induration.

Induration	Mean eGFR	Std. Deviation	No of Patients
Present	20.14	13.083	84
Absent	12.87	7.968	156

Mean eGFR of the patients with positive induration (>5 mm) was 20.14 ± 13 and those without induration (<5 mm) was 12.8 ± 8 with $p=0.003$, which was statistically significant i.e. cases with positive induration have higher eGFR values (Student t test).

Table 4 reveals induration related to hemodialysis.

Table 4 Induration related to hemodialysis.

No of hemodialysis	Induration	No Induration
0	33 (55.0%)	27 (45.0%)
1-2	27 (34.6%)	51 (65.4%)
>2	24 (23.4%)	78 (76.6%)
Total	84 (35.0%)	156 (65.0%)

Statistical analysis revealed significant ($p=0.03$) association between number of haemodialysis and induration i.e. more induration response in no hemodialysis group.

Discussion

Chronic kidney disease is considered as an immunocompromised state, given that T-cell, B-cell, and monocyte/macrophage function are all diminished [8]. These abnormalities are evidenced in part by the greater susceptibility to fungal and tuberculous infections. T-cell activation and proliferation are depressed, lymphokine production and antibody-dependent cell-mediated cytotoxicity are reduced, and there is increased suppressor cell activity, among other abnormalities [9]. In addition, B cell counts are decreased. Although total antibody production is not reduced, there is decreased IgG production in response to vaccination, probably related to impaired generation of antigen-specific helper T cells that are required for appropriate B-cell antibody synthesis in response to vaccination. Macrophages also do not function properly in uremic blood, with decreased interleukin (IL)-1 production [10].

Several studies have been done 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 is greater than or equal to 5 mm and 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 to a DTH response are considered to be anergic and are at the 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.

All the subjects were injected intradermal Candin antigen and evaluated for induration after 48 hrs. Among control group 58.8% individuals showed induration response with a mean induration of 5.3 mm. Among cases we recorded varied induration responses (≥ 5 mm) depending upon the stages of CKD patients. In stage 3, more than 50 % cases showed positive induration (≥ 5 mm), but however as the stage progressed; percentage of the patients with positive induration response went on decreasing for example 52% and 24% in stage 4 and 5 respectively.

Induration was significantly ($p=0.007$) more positive in stage 3 and 4 than in stage 5 which indicates that cellular immunity of CKD patients decreases as ESRD approaches. Mean eGFR of the patients with positive induration ($\geq 5\text{mm}$) was 20.14 ± 13 and those without induration ($<5\text{ mm}$) was 12.8 ± 8 with significant p value (0.0030), i.e. cases with positive induration have higher eGFR values. The result is in conformity with several studies; which showed defective cooperation between T cells and APCs (especially monocytes) leading to subnormal proliferation and interferon- γ and interleukin (IL)-2 production, and thus reduced cellular immunity and DTH response [11,12].

The number of circulating T-lymphocytes are reduced in HD and the reduction of T helper cells are more prominent than T suppressor cells. It was found that hemodialysis reduces the cellular immunity by functional impairment of T cells [13,14]. In our study induration response is significantly ($p=0.03$) more positive in no haemodialysis group which suggests that HD reduces immunity, in conformity with other studies as noted above.

Available studies reported that antigens of *Candida albicans* are useful in the assessment of diminished cellular immunity in persons infected with human immunodeficiency virus, moreover responses to DTH antigen [15,16] have also reported to have

prognostic values in patients with cancer [17] but it is for the first time Candin antigen has been used in CKD patients to assess DTH response with encouraging results; hence the matter needs further research.

The combination of immune system abnormalities in CKD leads to lower seroconversion rates, lower peak antibody titers, and more rapid decline of antibody levels [10,18,19]. Vaccines thus may be potentially less effective and provide less protection from infections among patients with kidney failure.

The study clearly states that by using Candin test we can single out in advance those patients who are in the state of deteriorating cellular immunity in order to provide additional therapy to improve quality of their lives and also vaccinate such patients with augmented regimens for providing extra protective cover for reducing the morbidity from various infections. However, adequate seroresponse has been documented with standard or augmented regimens for vaccinations against influenza, hepatitis B, pneumococcus, and varicella [10,19,20], but the clinical response to vaccination is not well understood. During this study period no CKD patient of stage 1 and 2 has been enrolled but if the Candin skin test had been done on such patients, encouraging results would have been got by protecting them with several infections.

References

- 1 Neovius M, Jacobson SH, Eriksson JK, Elinder CG, Hylander B (2014) Mortality in chronic kidney disease and renal replacement therapy: a population-based cohort study. *BMJ Open* 4: e004251.
- 2 Ortiz A, Covic A, Fliser D, Fouque D, Goldsmith D, et al. (2014) Epidemiology, contributors to, and clinical trials of mortality risk in chronic kidney failure. *Lancet* 383: 1831-1843.
- 3 Vermeiren AP, Hoebe CJ, Dukers-Muijers NHJ (2013) High non-responsiveness of males and the elderly to standard hepatitis B vaccination among a large cohort of healthy employees. *Clin Virol* 58: 262-264.
- 4 Middleton E, Reed CE, Ellis FE, Adkinson NF, Yunginger JW, Busse WW (1993) *Allergy Principles and Practice*. Mosby, Sr. Louis. 4th Ed., Vol II, pp. 963-982.
- 5 Bernstein IL (1988) Proceedings of the task force of guidelines for standardizing old and new technologies used for the diagnosis and treatment of allergy. *J Allergy Clin Immunol* 82: 487-526.
- 6 KDIGO (2013) *Kidney International Supplements* 3: 5-14.
- 7 Allergmed Laboratories Inc. San Diego, CA 92111.
- 8 Chatenoud L, Herbelin A, Beaurain G, Descamps-Latscha B (1990) Immune deficiency of the uremic patient. *Adv Nephrol Necker Hosp* 19: 259-274.
- 9 Kausz A, Pahari D (2004) The Value of Vaccination in Chronic Kidney Disease. *Seminars in Dialysis* 17: 9-11.
- 10 Johnson D, Fleming S (1992) The use of vaccines in renal failure. *Clin Pharmacokinet* 22: 434-446.
- 11 Brinkkoetter PT, Marinaki S, Gottman U, Fleckenstein S, Stump C, et al. (2005) Altered CD46-mediated T cell co-stimulation in hemodialysis patients. *Clin Exp Immunol* 139: 534-541.
- 12 Meier P, Dayer E, Ronco P, Blanc E (2005) Dysregulation of IL-2/IL-2R system alters proliferation of early-activated CD4+ T cell subset in patients with end-stage renal failure. *Clin Nephrol* 63: 8-21.
- 13 Litjens NH, van Druningen CJ, Betjes MG (2006) Progressive loss of renal function is associated with activation and depletion of naive T lymphocytes. *Clin Immunol* 118: 83-91.
- 14 Yoon JW, Gollapudi S, Pahl MV, Vaziri ND (2006) Naive and central memory T-cell lymphopenia in end-stage renal disease. *Kidney Int* 70: 371-376.
- 15 Blatt SP, Hendrix CW, Butzin CA, Freeman TM, Ward WW, et al. (1993) Delayed-type hypersensitivity skin testing predicts progression to AIDS in HIV- Infected patients. *Ann Int Med* 119: 177-183.
- 16 Colebunders RL, Lebughe I, Nzila N, Kalunga D, Francis H, et al. (1989) Cutaneous delayed-type hypersensitivity in patients with human immunodeficiency virus infection in Zaire. *J Acq Immune Def Synd* 2: 576-578.
- 17 Ahmed AR, Blose DA (1983) Delayed-type hypersensitivity skin testing. A review. *Arch Dermatol* 119: 934-945.
- 18 Beaman M, Michael J, MacLennan IC, Adu D (1989) T-cell-independent and T-cell-dependent antibody responses in patients with chronic renal failure. *Nephrol Dial Transplant* 4: 216-221.
- 19 Fuchshuber A, Kuhnemund O, Keuth B, Luticken R, Michalk D, et al. (1996) Pneumococcal vaccine in children and young adults with chronic renal disease. *Nephrol Dial Transplant* 11: 468-473.
- 20 Charest AF, McDougall J, Goldstein MB (2000) A randomized comparison of intradermal and intramuscular vaccination against hepatitis B virus in incident chronic hemodialysis patients. *Am J Kidney Dis* 36: 976-982.