ORIGINAL RESEARCH

Differentiation of Oral Candida Species in Chronic Renal Failure Patients Undergoing Hemodialysis

Jabeena Gowher[1], K. Karpagaselvi[2], Anjum Baker[3]

Abstract

Background: Oral candidiasis occur as an opportunistic infection. The transition of candida from commensal to pathogen is often associated with immune- compromised chronic renal patients receiving hemodialysis. Candida species identification and differentiation is important for treatment in these patients.

Aim: To differentiate candida species present in the oral cavity of chronic renal failure patients undergoing hemodialysis.

Material and Methods: A total of 120 individuals with study group (n=60) of chronic renal failure patients undergoing hemodialysis (CRF with HD) and control group (n=60) healthy individuals, were studied. Salivary samples were cultured for candida species using Sabouraud's Dextrose Agar (SDA) and CHROM agar culture media, for the growth of candida species in 24, 48 to 72 hours at 37°C. Colonies were counted and quantitatively expressed as colony forming units/milliliter (CFU).

Results: Positive candidal growth was seen on SDA and CHROM agar medium among CRF with HD and Control Groups, candida species were present in 55 (91.6%) and absent in 5(8.3%) and 57(95%) and absent 3(5%) in individuals respectively. Candida species differentiation in CRF with HD and Control groups were C. albicans (green colonies), C. Kruzei (pink colonies) and C. Tropicalis (blue colonies) were 46(81.6%), 6(10.0%), 2(3.3%) in CRF with HD cases while 45(75%) 11(18.3%) and 0(0%) in control cases respectively.

Conclusion: Isolation and differentiation of candida was highly significant ($p<0.05$) in chronic renal failure patients undergoing hemodialysis than healthy individuals.

Keywords: Chronic renal failure, Hemodialysis, Candida species, Sabouraud's dextrose agar, CHROM agar

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Introduction

Chronic renal failure (CRF) is emerging as a major health problem and is a considerable cause for morbidity and mortality worldwide[1]. CRF is defined by the National Kidney Foundation as Kidney Disease Outcomes Quality Initiative as "structural and functional abnormalities of the kidney with or without decrease in Glomerular Filtration Rate (GFR) manifesting either by pathologic abnormalities or other markers of kidney damage including abnormalities in the composition of blood or urine."The presence of GFR less than 60ml/min/1.73m^2 body surface area for more than 3 months with or without other signs of kidney damage is a sign of CRF.[3] Hemodialysis is indicated when residual Glomerular Filtration Rate (GFR) falls below 10 ml/min /1.73 m^2 body surface area or when the patient loses the energy to sustain normal daily work and activity. When symptoms such as nausea, vomiting, anorexia, fatigability, diminished sensorium and signs like pericardial friction rub, refractory pulmonary edema, and metabolic acidosis of uremia occur, dialysis treatment is urgently indicated. Patients presenting with CRF are at high risk of developing oral health complications such as thinning of pulp chamber, enamel abnormalities, xerostomia, premature tooth loss, increased frequency of calculus and periodontal disease.[4]

[1] Department of Oral Pathology, KGF College of Dental Sciences, KGF-563115 Karnataka; [2] Department of Oral Pathology, Vydehi institute of Dental sciences, Bangalore-560066, Karnataka; [3] Department of Oral pathology, Al Azhar Institute of Dental Sciences, Thodupuzha, Kerala-685605.

Corresponding author: Jabeena Gowher, Department of Oral Pathology, KGF College of Dental Sciences and Hospital, Karnataka-563115, India, Email: drjgowher@gmail.com

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It is well known that these patients are immuno-compromised and their salivary flow tends to slow. When the immune system is suppressed, microbial agents of normal oral flora have an opportunity to become pathogenic, which causes infection and destruction of the oral cavity. A depressed immune system predisposes opportunistic infection by candida species. The transition of candida from commensal to pathogen is often associated with predisposing factors like prolonged antibiotic use, viral infections, immune-compromised patients and patients receiving hemodialysis and renal transplantation.[5]

Dialysis patients are at increased risk of mortality due to systemic fungal infection compared to the general population. Candida species account for 8–15% of all nosocomial infections of which patients with dialysis dependency (those on continuous renal replacement therapy) are more prone to fungal infections than those non- dialyzed. United States renal data system (USRDS) described candidiasis as the major causative factor of fungal infection in 79 % of chronic dialysis patients.[5]

Candida albicans are recovered from 60% of dentate patients over the age of 60. The main factors which increases susceptibility to oral candidiasis are, immunodeficiency, nutritional deficiency, malignancies, dental prosthesis, high carbohydrate diet, malabsorption, old age, poor oral hygiene, heavy smoking, antibiotics, diabetes, dysplasia, atopy and xerostomia.[6] The important subspecies are C.albicans, C.tropicalis, C.glabrata, C.pseudotropicalis, C.guillierimondii, C.krusei, C.lusitaniae, C.parapsilosis, and C.stellatoidea. C.albicans, C.glabrata, and C.tropicalis represent more than 80% of isolates from clinical infection. C. albicans is a normal commensal of the mouth and generally does not cause infection in healthy people. The most discrete lesion represents conversion from benign colonization to pathological overgrowth[7]. We attempted to isolate and differentiate candida species present in the oral cavity of chronic renal failure patients undergoing hemodialysis.

Materials and Methods

This study was conducted at Department of Oral Pathology, Vydehi Institute of Dental Sciences & Research Centre, Bangalore. Patients who were clinically diagnosed cases of chronic renal failure patients undergoing hemodialysis reported to the department of Nephrology at Vydehi medical college hospital, Bangalore were considered for the study. Ethical clearance was obtained from the institutional ethical committee for human experimentation as per standard guidelines and informed consent was obtained from all the individuals.

It is a hospital based case control study consisting of 120 patients divided into, 60 patients with chronic renal failure undergoing hemodialysis (CRF with HD) and 60 healthy individuals as controls. Detailed Oral examination of all the individuals were carried out using diagnostic instruments. All Chronic renal failure patients undergoing hemodialysis and healthy individuals as controls were included in the study. Patients subjected to radiotherapy / chemotherapy, Diabetes mellitus. Patients on any long term medications like systemic corticosteroids, antibiotics, and immunomodulants. Patients wearing dentures were excluded from the study.

Microbiological method and species identification

Collection of Unstimulated whole saliva - Navazesh's method[9,10,14]

The subjects were asked to refrain from intake of any food, beverage, smoking or chewing gum for one hour before the sample collection. The subjects were seated on a dental chair, asked to rinse their mouth with distilled water and then to relax for five minutes. They were instructed to minimize movements and asked to lean the forehead over a funnel and a test tube kept below it. The subjects were asked to keep their mouth slightly open and allow

Table 1: Evaluation of Candida in Sabouraud's dextrose agar media among the study groups

Study Groups	Candida in Sabouraud's dextrose agar media		
	Present (%)	Absent (%)	P value
CRF with HD	55 (91.6)	5(8.3)	0.0001(highly significant)
Controls	4(6.7)	56 (93.3)	

Table 2: Evaluation of Candida in CHROM agar media among the study groups

Study Groups	Candida in CHROM agar media		
	Present (%)	Absent (%)	P value
CRF with HD	57(95)	3(5)	0.0001(Highly significant)
Controls	4(6.7)	56(93.3)	

Table 3: Comparison of number & percentage of colonies in CRF with HD & Controls in CHROM agar media

Growth of the colonies	CRF with HD		Controls		P value
	n	%	n	%	
No growth	3	5	4	6.7	0.0001 (highly significant)
High	35	58.3	0	0.0	
Medium	13	21.6	0	0.0	
Low	9	15	56	93.3	
Total	60	100.0	60	100.0	

Table 4: Comparison of number of colonies, color colonies and percentage of candida species in CRF with HD & Control groups.

Candida species differentiation	CRF with HD		Controls		P value
	n	%	n	%	
C.Albicans (Green colonies)	49	81.6	45	75	0.0001 (highly significant)
C. Kruzei (Pink colonies)	6	10.0	11	18.3	
C. Tropicalis (Blue colonies)	2	3.3	0	0.0	
C. Glabratta (white colonies)	Nil	Nil	Nil	Nil	
C.krusei and C. Tropicalis	Nil	Nil	Nil	Nil	
C. Kruzei and C. Glabartta	Nil	Nil	Nil	Nil	

saliva to drain into the tube for five minutes.

Culturing for candida: The collected salivary sample was centrifuged at 2000 rpm for 15 minutes and the sediment was obtained for fungal culture. A loop of sample was inoculated on SDA plates and incubated at 37^{0}C. The colonies were periodically checked for the growth at 0, 12, 24, 48 and 72 hours.

Gram stain and Germ tube testing:[8,11,] The colonies grown on the plates were subjected to Grams stain. The species which showed positivity to candida were again subjected to germ tube test. Germ tube test is the rapid screening procedure for observing germ tubes formation, identifies and differentiates C.albicans from other candida species. The culture of candida species was treated with 1 ml of sterile mammalian (fetal- bovine, sheep or normal human) serum & incubated at 37^{o}c for 2-4 hours. After incubation,a drop of suspension was examined on the glass slide under the microscope for the presence of germ tube.[7,9,10,11.16]

1ml of human serum taken in a test tube + 1 loop of candida colony were mixed and incubated for 2 hours at 37^0 Celsius. A drop of it is taken on sterile slide and coverslip is placed and observed under microscope for presence of germ tube.[8,15]

Candida species differentiation: The fungal colonies that showed positivity for germ tube formation were streaked on CHROM agar plates by using sterile plastic inoculating loops for candida species differentiation based on color and morphology was done. The colonies were periodically checked for the growth at 0, 12, 24 and 48 hours.

Identification of colonies:[17] Candida albicans -Green colonies Candida glabrata -White Candida kruzei - Pink colonies, Candida Tropicalis - Purple or blue colonies.

Counting of candida colonies: The number of colonies thus obtained were counted and quantitatively expressed as number of colonies obtained per ml of saliva. i.e. Colony Forming Units/ml (CFU)[1].

Statistical Analysis:

Data obtained were computed on Microsoft excel sheet. Statistical analysis was carried by using Statistical package for social sciences (SPSS version 17.0, Chicago, USA)using Chi-square has been used to find the significance of study parameters on categorical scale between the groups. P value: <0.05 was considered statistically significant.

Results

Total number of subjects participated in the study were 120 and were classified into two groups, 60 patients with chronic renal failure undergoing hemodialysis (CRF with HD) and 60 healthy individuals as controls. Overall the patient's age ranged between 20 to 70 years of age. Distribution of patients in each age group showed the highest number of patients in the CRF with HD group were observed in the age range between 41-50 years, with the least number of patients noted in 21-30 years age group. The mean age in the present study sample was 47.73±13.60 in CRF with HD group and 37.25± 13.15 in the control group. The samples were age matched with a p value <0.001.

The gender distribution of the patients in the CRF with HD group constituted a majority of 62% males & 38% females when

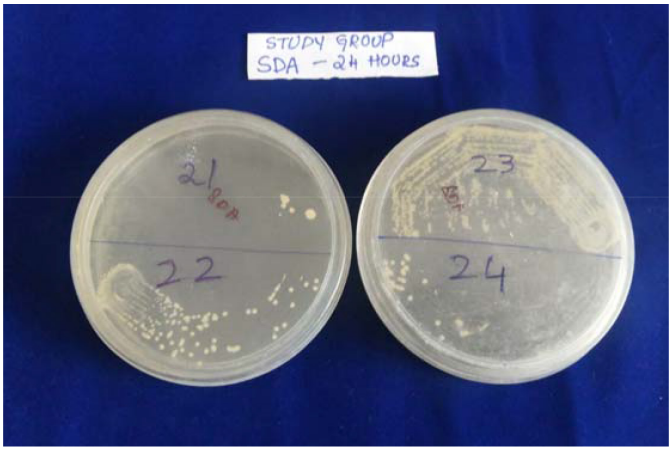

Fig 1: The Sabouraud's dextrose agar (SDA-24hrs) media showing creamy, white colonies representing candida species in CRF with HD cases.

Fig. 2: Hi CHROM candida differential agar media (72hrs) showing green color colonies representing Candida albicans, Pink color colonies representing Candida kruzei and Blue color colonies representing Candida tropicalis in CRF with HD cases.

Fig. 3: Hi CHROM candida differential agar media (48hr) showing green color colonies representing Candida albicans, Pink color colonies representing Candida kruzei in CRF with HD cases.

compared to Controls 53% males and 47% females respectively. Samples were gender matched with p<0.001.

On evaluation on SDA medium among CRF with HD and Control Groups. Candida was present in 55 (91.6%) and absent in 5(8.3%) individuals respectively. On statistical analysis of intergroup comparison, an extremely significant difference (p<0.0001) was noted. [Table 1, Fig 1].

On evaluation on CHROM agar media among CRF with HD and Control groups, candida species were present in 57(95%) and absent in 3(5%) individuals respectively. [Table 2].

Comparing the number of colonies growth of candida in two groups revealed a significantly higher value of colonies in CRF with HD cases when compared Control group. Based on the number of colonies were divided into No growth, high, medium, and low growth. The values were 3(5%), 35(58.3%), 13(21.6%), 9(15%) in CRF with HD cases and 4(6.7%), 0(0%), 0(0%), 56(93,3%) respectively. The highest value obtained in CRF with HD group were 94.9% (>400 colonies) -CFU /ml of saliva, where majority of 57 cases fall in to this values, whereas highest value obtained in control group were 56(93.3%) (<250 colonies)-CFU /ml of saliva [Table 3]. Thus, colonies growth showed significant p value <0.0001. Candida species differentiation based on number of colonies, color colonies and percentage of candida species in CRF with HD & Control groups were C. albicans (green colonies), C. Kruzei (pink colonies) and C. Tropicalis (blue colonies) were 46(81.6%), 6(10.0%), 2(3.3%) in CRF with HD cases while 45(75%) 11(18.3%) and 0(0%) in control cases respectively [Table 4, Figs. 2 and 3]. However CRF with HD and control group did not show any combination of candida species. All the other forms of fungi except candida were considered as contamination. Contamination in the form of fungal molds were present in 6(10%) and 8(13.3%) in CRF with HD and control groups.

Discussion

Chronic renal failure (CRF) is characterized by progressive and irreversible loss of kidney functions over a period of years, which results in chronic deterioration of nephrons and makes it necessary to recruit extra renal blood filtering technique like hemodialysis.[2] Candida species account for 8–15% of all hospital acquired infections of which patients with dialysis dependency are more prone to fungal infections than those non-dialyzed When the immune system is suppressed, microbial agents of normal oral flora have an opportunity to become pathogenic, which causes infection and destruction of the oral cavity.[5,15,18,19]

In the present study, the age of presentation of CRF patients ranged from 20 years to 70 years with a mean age of 40-50 years in the study group and 30-50 years in the control group. Our findings were in concordance with Ahamaidieh et al age group of CRF patients on hemodialysis the range was between 40-50 years with a mean age of 55.8 ±14.4 years.[18]

The gender distribution in CRF with HD cases were 62% males and 38 % females and controls were 53% males and 47% females respectively. Our findings were in agreement with Jenabian et al, aimed to assess the haemodialysis patients with periodontal status approximately with similar gender distribution.[20] The collected swab sample was inoculated primarily on SDA medium, followed by CHROM agar (a differential medium) for speciation. The same combination of culture media were used by Nadeem et al[21] and Odds and Bernaerts[22].

Candida species differentiation based on number of colonies, color colonies and percentage of candida species in CRF with HD and Control groups were C. albicans (green colonies), C. Kruzei (pink colonies) and C. Tropicalis (blue colonies) were 46(81.6%), 6(10.0%), 2(3.3%) in CRF with HD cases while 45(75%) 11(18.3%) and 0(0%) in control cases respectively. Similar findings were reported by Manikanandan and Amsath, in which C.albicans 70%, followed by C.galabrata 16.6%, C.krusei 6.7%, C.tropicalis 6.7% was found respectively.[17]

Our findings revealed comparing the number of colonies growth of candida in two groups revealed a significantly higher value of colonies in CRF with HD cases when compared Control group. Based on the number of colonies were divided into No growth, high, medium, and low growth. The highest value obtained in CRF with HD group were 94.9% (>400 colonies) -CFU /ml of saliva, where majority of 57 cases fall in to this values, whereas highest value obtained in control group were 56(93.3%) (< 250 colonies)-CFU /ml of saliva. Thus, colonies growth showed significant p value <0.0001.

Our findings were in agreement with Epstein et al who performed colony count of Candida albicans in saliva in which statistical analysis showed significant difference between colony count in saliva from patients with chronic candidiasis (p<0.002) which indicates individuals <400 CFU/ml carriers & those with >400CFU/ml were having acute or chronic candidiasis.[23] This was in concordance with the study conducted by Manikanandan and Amsath.[17] However in the present study comparing the colony growth in two groups revealed a significantly higher value CRF with HD cases when compared to controls group. Our results showed that there was a significant increase in Candida albicans in hemodialysis patients as previously attributed by some authors.[17,18] As reported by Yohei T et al, patients on hemodialysis with renal disease tend to have significantly more candida albicans (p<0.05) than healthy control group. Similarly in the present study the patients on hemodialysis had significantly more Candida albicans (p<0.05).

Conclusion

Differentiation of candida was more significant in CRF patients with hemodialysis, than in healthy individuals, which might be due to immunodeficiency. Considering the risk of colonization progress to fungal infections, severe and often fatal. This study may help to elucidate the influence of oral candida in the quality of life of patients with CRF. The importance of anti- mycotic therapy with other treatment and ideal oral hygiene instructions with counseling has to be emphasized towards elimination of fungal infections from CRF patients on hemodialysis.

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