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ESTUDO DO ESTADO DE SAÚDE ORAL EM DOENTES TRANSPLANTADOS RENAIS

STUDY OF ORAL HEALTH STATUS IN RENAL TRANSPLANT RECIPIENTS

DISSERTAÇÃO DE CANDIDATURA AO GRAU DE DOUTOR
APRESENTADA À FACULDADE DE MEDICINA DENTÁRIA
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Aos meus pais.
A minha maior gratidão pelo vosso amor e esforço.

Ao Óscar e à Amélia,
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Abstract

Kidney disease is a worldwide public health problem associated with an increased cardiovascular risk and all cause mortality. End-stage renal disease is defined by the cessation of effective kidney function and the beginning of renal replacement therapy, such as hemodialysis, peritoneal dialysis or kidney transplantation. The renal replacement therapy of choice for end-stage renal disease is kidney transplantation. It is widely accepted that kidney transplantation improves quality and length of life, and costs less than dialysis.

Kidney transplantation demands immunosuppressant therapy to avoid renal transplant rejection. The recent expansion in immunosuppressive agents licensed for use in renal transplant recipients has dramatically increased the number of potential drug combinations available to the clinician.

In current literature, the comparison of calcineurin inhibitors effects on oral health is of great interest; however fewer studies are found concerning inhibitors mTOR (mammalian target of rapamycin) effects on oral health.

Oral manifestations of CKD are common. In chronic dialysis patients dental diseases are considered less prevalent and periodontal diseases are more common. In renal transplant recipients drug induced complications and infections are the most important. In literature it is suggested that the inflammatory or infectious local burden associated to periodontal disease can predispose to bacteremia and it can lead to an increased cardiovascular disease risk.

The aim of the present study was to evaluate the differences in the oral health status of renal transplant recipients (RTRs) administrated with Cyclosporin A (CsA), Tacrolimus (Tac) or Everolimus (ERL), and compare it with patients on hemodialysis (pre-transplant) and healthy controls (living kidney donor). Furthermore, oral microbiota, salivary and serum biochemical parameters will be studied when alterations in oral health status are observed among groups.

A total of 88 RTRs have participated in the study, 29 of these were RTRs receiving CsA, 36 were RTRs receiving Tac and 23 were RTRs receiving ERL. Additionally, 13

LKDs and 23 HD patients were used as control groups. Demographic and pharmacological data were recorded in all groups. Oral health status was assessed through the analysis of oral hygiene habits and oral symptoms, teeth evaluation (visible plaque index and DMFT index), periodontal evaluation (bleeding on probing, clinical attachment level), gingival enlargement, gingival index, salivary pH and flow rate.

Oral fungi colonization was assessed by isolation and quantification for all studied groups. Saliva composition was assessed through the quantification of its parameters using an automatic analyzer. The relation between salivary and serum biochemical parameters was studied by calculating a ratio.

Renal transplant recipients receiving Tac were younger than CsA, ERL, HD and LKD-groups, whereas the prevalence of female gender was higher in LKDs group. No differences were found among the studied groups concerning smoking habits.

When RTRs were compared to HD and LKD groups no differences were found among the three groups concerning oral hygiene habits and oral symptoms. No differences were observed in teeth and periodontal evaluation. Although unstimulated and stimulated saliva pH did not differ among groups, unstimulated and stimulated saliva flow rates were lower in HD patients than in RTRs and LKD groups. Concerning oral fungi colonization no differences were found among the studied groups.

When RTRs receiving CsA, Tac or ERL were compared, no differences were found among the three groups concerning oral hygiene habits and oral symptoms. Additionally, no differences were found in teeth and periodontal evaluation, except for bleeding on probing which presented itself as lower in ERL than in CsA and Tac groups. No differences were observed in unstimulated and stimulated saliva flow rate and pH among the three groups.

Saliva composition revealed differences when HD patients, RTRs and LKDs were compared. Patients on hemodialysis presented higher levels of potassium, urea, creatinine, lipid profile (LDL cholesterol, triglycerides), aspartate aminotransferase and alkaline phosphatase. In what concerns RTRs the main

results were the lower levels of immunoglobulins A and G when compared with HD patients and healthy controls. The saliva/serum ratio revealed itself as an important indicator of biochemical parameters variation, namely for potassium, phosphate, urea, creatinine, and lipid profile.

Renal transplant recipients receiving ERL had less periodontal inflammation and may be better protected for the development of periodontal disease when compared to RTRs receiving Tac or CsA.

Patients on hemodialysis had less oral cleaning than RTRs or LKDs. Concerning saliva composition, the lower salivary secretion rate and the potassium derived from serum could be responsible for the higher levels of salivary potassium observed; the lower salivary uric acid could lead to lower protection towards oxidative damage; and higher salivary levels of enzymes, namely the aspartate aminotransferase and alkaline phosphatase, could be associated to more tissue damage in oral cavity of these patients.

Renal transplant recipients had less immunological protection in oral surfaces due to the lower salivary immunoglobulin A. Besides that, the higher levels of potassium in this group could have been determined by the salivary glands activity and not by serum.

In RTRs receiving Tac their higher salivary C-reactive protein levels could be considered an indicator of a higher inflammatory activity in oral tissues. In addition, RTRs receiving Tac and ERL could be endowed with better conditions to maintain dental structure, than RTRs receiving CsA.

Saliva could be an alternative tool for monitoring the oral and systemic health and a sample substitute to serum in some biochemical parameters; its simplicity, convenience and non-invasiveness are significant advantages.

The previous conclusions reinforce that an adequate oral health care may be particularly recommended for RTRs and HD patients; this is essential for their general health and well-being.

Resumo

A doença renal crónica é considerada um problema de saúde pública em todo o mundo, e está associada a um maior risco de morbilidade e mortalidade. É amplamente aceite que o transplante renal melhora a qualidade e a esperança de vida dos indivíduos que padecem desta doença.

No transplante renal a terapêutica imunossupressora é necessária para evitar ou prevenir a rejeição do órgão. Os agentes imunossupressores têm registado um incremento substancial no seu número o que potencia as combinações farmacológicas disponíveis. Na literatura recente, a comparação dos efeitos dos inibidores da calcineurina (ciclosporina A e tacrolimus) na saúde oral é de grande interesse. Contudo, poucos estudos são encontrados relativamente aos inibidores seletivos de mTOR - alvo da rapamicina de mamíferos (everolimus).

As manifestações orais na doença renal crónica são comuns. Enquanto que nos pacientes em diálise as doenças dentárias são consideradas menos prevalentes e as doenças periodontais são mais comuns, nos doentes transplantados renais as complicações induzidas por fármacos e as infeções são as mais frequentes. Na literatura sugere-se que a carga inflamatória local e/ou infeciosa associada à doença periodontal pode predispor à bacteriemia e a um aumento do risco de doença cardiovascular.

O presente estudo tem como objetivos avaliar as diferenças no estado de saúde oral de doentes transplantados renais medicados com Ciclosporina A (CsA), Tacrolimus (Tac) e Everolimus (ERL), como imunossupressores, e compará-los com doentes em hemodiálise (pré-transplante) e dadores vivos de rim (controlos saudáveis). Para além disso, sempre que forem observadas alterações no estado de saúde oral entre os grupos estudados, a microbiota oral e os parâmetros bioquímicos na saliva e no sangue serão avaliados.

No estudo participaram 88 transplantados renais, sendo 29 medicados com CsA, 36 com Tac e 23 com ERL. Adicionalmente, 23 doentes em hemodiálise e 13 dadores vivos de rim participaram como grupos controlo. Para todos foram registadas variáveis demográficas e farmacológicas. O estado de saúde oral foi

avaliado através da análise dos hábitos de higiene oral e sintomas orais, do exame dentário (índice de placa e no índice CPO) e periodontal (hemorragia pós-sondagem, nível clínico de inserção), do aumento gengival e índice gengival, da análise da taxa de fluxo e do pH salivar.

A presença de fungos na cavidade oral e a sua quantificação foram determinados para cada um dos grupos. A composição da saliva foi avaliada pela quantificação dos seus parâmetros usando um analisador automático. A relação dos parâmetros bioquímicos na saliva e no sangue foi avaliada através da determinação de um quociente.

Os transplantados renais medicados com Tac eram mais jovens que os transplantados medicados com CsA ou ERL, e do que os doentes em hemodiálise e controlos saudáveis. A prevalência de mulheres era maior nos dadores vivos de rim, enquanto que a prevalência de homens era maior nos transplantados renais. Não foram encontradas diferenças estatisticamente significativas no que respeita aos hábitos tabágicos entre os grupos. Quando os transplantados renais foram comparados com os doentes em hemodiálise e com controlos saudáveis não foram observadas diferenças nos hábitos de higiene oral e sintomas orais, e também não foram observadas diferenças na avaliação dentária e periodontal. Embora não tenham sido observadas diferenças no pH da saliva, a taxa de fluxo da saliva não estimulada e estimulada foi menor nos doentes em hemodiálise do que nos transplantados renais e controlos saudáveis. No que diz respeito à presença de fungos na cavidade oral não foram observadas diferenças nos grupos estudados.

A comparação entre os transplantados renais medicados com CsA, Tac e ERL não revelou diferenças no que respeita aos hábitos de higiene oral e sintomas orais. O mesmo se verificou na avaliação dentária e periodontal, com exceção do índice de hemorragia pós-sondagem que se apresentou menor no grupo de transplantados renais medicados com ERL do que nos grupos medicados com CsA e Tac. Não foram observadas diferenças no pH e taxa de fluxo salivar entre os três grupos de transplantados.

A composição da saliva revelou diferenças quando foram comparados os doentes em hemodiálise com os transplantados renais e controlos saudáveis. Os doentes

em hemodiálise apresentaram níveis mais elevados de potássio, ureia, creatinina, perfil lipídico (colesterol LDL e triglicerídeos), e enzimas, aspartato aminotransferase e fosfatase alcalina. Os transplantados renais apresentaram níveis mais baixos de imunoglobulinas A e G quando comparados com os doentes em hemodiálise e controlos saudáveis. O quociente dos parâmetros bioquímicos na saliva e no sangue revelou-se como um importante indicador para o potássio, fosfato, creatinina, ureia e perfil lipídico.

Os doentes transplantados renais medicados com ERL têm menos inflamação periodontal e parecem estar melhor protegidos para a doença periodontal, quando comparados com os transplantados renais medicados com CsA e Tac.

Nos doentes em hemodiálise a capacidade de remoção das bactérias e resíduos pela secreção de saliva foi menor. Relativamente à composição da saliva, a menor taxa de secreção salivar e o potássio derivado do sangue poderão estar na origem dos níveis elevados de potássio observados; os níveis mais baixos de ácido úrico podem condicionar uma menor proteção antioxidante; e níveis mais altos das enzimas, aspartato aminotransferase e fosfatase alcalina, podem ser indicadores de maiores danos nos tecidos orais destes doentes, quando comparados com os doentes transplantados renais e os controlos saudáveis.

Os doentes transplantados renais apresentaram uma menor proteção imunológica nas mucosas orais devido aos baixos níveis salivares de imunoglobina A. Os níveis elevados de potássio na saliva para este grupo podem ter sido determinados pela atividade das glândulas salivares e não pela influência do sangue. Uma maior atividade inflamatória nos tecidos orais pode existir nos doentes transplantados renais medicados com Tac, considerando os elevados níveis de proteína C reativa detetados na saliva deste grupo. Considerando também os elevados níveis de fosfato encontrados na saliva dos doentes transplantados renais medicados com Tac e ERL, estes podem estar dotados de melhores condições para proteger a sua estrutura dentária do que os transplantados medicados com CsA.

A saliva pode ser uma ferramenta útil para monitorizar a saúde oral e sistémica, e também uma amostra alternativa ao sangue para mensuração de alguns

parâmetros bioquímicos. A simplicidade da sua colheita, a sua conveniência para o paciente e o facto de não ser invasiva, são vantagens deveras importantes.

As conclusões anteriores vêm reforçar que os cuidados médicos dentários nos doentes transplantados renais e doentes em hemodiálise são particularmente recomendados, sendo mesmo indispensáveis para o seu estado de saúde geral e bem-estar.

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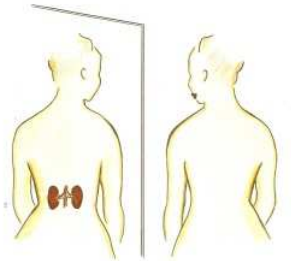
List of Acronyms and Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
ALP	Alkaline Phospatase
aPTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
BCG	Bromocresol Green
BOP	Bleeding on Probing
BUN	Blood Urea Nitrogen
CAL	Clinical Attachment Loss
CAN	Chronic Allograft Nephropathy
CFU	Colony Forming Units
CKD	Chronic Kidney Disease
CMV	Cytomegalovirus
CNI	Calcineurin inhibitors
CP	Chronic Periodontitis
CRP	C-reactive protein
CsA	Cyclosporine A
CVD	Cardiovascular Disease
DGKC	Deutsche Gesellschaft für Klinische Chemie
DIC	Disseminated Intravascular Coagulation
DMFT	Decayed, Missing and Filled Teeth
DOPPS	Dialysis Outcomes and Practice Pattern Study
ERL	Everolimus
ESRD	End-stage renal disease
GE	Gingival Enlargement
GFR	Glomerular Filtration Rate
GI	Gingival Index
GO	Gingival Overgrowth
HD	Hemodialysis
HDL	High-Density Lipoprotein

HIV	Human Immunodeficiency Virus
HPT	Hyperparathyroidism
IFCC	International Federation of Clinical Chemistry
IgAS	Immunoglobulin A
IgG	Immunoglobulin G
ITS	Internal transcriber spacer
K/DOQI	Kidney Disease Outcomes Quality Initiative
LDH	L-Lactate dehydrogenase
LDL	Low-Density Lipoprotein
Ig	Immunoglobulin
LKD	Living kidney donors
MMF	Mycophenolate Mofetil
mTOR	Mammalian Target of Rapamycin
NFK	National Kidney Foundation
PAP	Prostatic Acid Phosphatase
PCR	Polymerase Chain Reaction
PD	Periodontal Diseases
PMN	Polymorphonuclear Leukocytes
PTH	Parathyroid Hormone
ROS	Reactive Oxidative Species
RRT	Renal Replacement Therapy
RTR	Renal Transplant Recipient
SD	Standard Deviation
s-IgA	Secretory Immunoglobulin A
SPSS	Statistical Package for Social Sciences
SRL	Sirolimus
Tac	Tacrolimus
TOR	Target of Rapamycin
UTI	Urinary Tract Infections
VPI	Visible Plaque Index

Chapter I – Introduction



1 Chronic kidney disease

Kidney disease is a worldwide public health problem recognized as a common condition that is associated with an increased risk of cardiovascular disease and chronic renal failure, with poor outcomes and high cost. [1]

Kidneys are two structurally complex organs that evolved to regulate several important functions such as: fluid volume and maintenance of acid/base balance of plasma, excretion the waste products of metabolism such as nitrogenous waste, regulation of body water and salt, secretion of a variety of hormones and autacoids and drug metabolism. [1-3]

Under normal physiological conditions, 25% of the circulating blood perfuses the kidney each minute. The blood is filtered through a complex network of tubules and glomerular capillaries, resulting in the ultrafiltrate, the precursor of urine. [3, 4]

Progressive kidney disease can result in reduced function and might affect several organ systems. Resultant anemia, abnormal bleeding, electrolyte and fluid imbalance, hypertension, drug intolerance, and skeletal abnormalities might affect the practice of dentistry. [1, 2]

Additionally, patients who have severe progressive disease may require artificial filtration of the blood through dialysis or kidney transplantation. These patients have become important in dentistry because of the growing number that survives renal failure due to renal dialysis or transplantation. [1, 2]

The Kidney Disease Outcomes Quality Initiative (K/DOQI) of the National Kidney Foundation (NKF) defines chronic kidney disease (CKD) as either kidney damage or a decreased glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m^2 for 3 or more months [5]. Whatever the underlying etiology, the destruction of renal mass with irreversible sclerosis and loss of nephrons leads to a progressive decline in GFR. The different stages of CKD form a continuum in time. In 2002, K/DOQI published its classification of the stages of CKD, as follows: stage 1, kidney damage with normal or increased GFR (>90 mL/min/1.73 m^2); stage 2, mild reduction in GFR (60-89 mL/min/1.73 m^2); stage 3, moderate reduction in GFR (30-59 mL/min/1.73 m^2); stage 4, severe reduction in GFR (15-29 mL/min/1.73 m^2); and stage 5, kidney failure (GFR < 15 mL/min/1.73 m^2 or dialysis). [4, 5]

The stage of CKD provides substantial prognostic and diagnostic information about outcomes, progression to end-stage renal disease (ESRD) and mortality [6, 7]; occurrence of intercurrent morbidity, ischemic heart disease, stroke and peripheral vascular disease [8, 9]; and it is predictive of complications prevalence associated with impaired kidney function, anemia, bone disease, and nutritional and functional status. [4]

Chronic kidney disease is associated to several complications, such as alteration of GFR [5]; proteinuria [10]; decreased quality of life, namely, difficulty on walking [5] and hemoglobin variation [11]; and outcomes, 5-year end stage renal disease rate, 5-year mortality rate, 3-year cardiovascular disease rate [6]. It is also related to risk factors, like cardiovascular, as hypertension [5], diabetes [10] and C-reactive protein [12]; nutritional, as albumin [12] and bicarbonate [12]; and bone disease, as phosphate [5, 13], calcium [5, 13], 25(OH)-vitamin D [13] and parathormone. [4, 13]

1.1 End-stage renal disease

End-stage renal disease is defined by the cessation of effective kidney function and the beginning of renal replacement therapy (RRT), such as hemodialysis (HD), peritoneal dialysis or kidney transplantation. The cessation of effective kidney function refers to bilateral, progressive, chronic deterioration of nephrons, the functional unit of the kidney. [3, 4, 14]

In the last 3 decades, an epidemic of ESRD, initially attributed to the dissemination and adoption of RRT, has occurred in both industrialized and developing countries [15, 16] and has been responsible for an increase in life expectancy. Epidemic ESRD has a substantial impact in public health. [4]

Patients treated for ESRD, when compared with individuals matching age-, gender- and ethnicity, have a shorter life expectancy. Furthermore, treatment is punctuated by frequent hospitalizations, progressive disability and worse quality of life. [4, 17, 18]

1.2 Epidemiology

1.2.1 Incidence and prevalence in Portugal

In Portugal there are 900 000 patients with kidney disease, and 1 in each 10 individuals suffers from CKD. Every year, are recorded 2 500 new cases of ESRD and there are at this time 16 000 patients with the most severe form of CKD, undergoing dialysis (about 10 000) or kidney transplant (6 000). [19]

1.2.2 Etiology

ESRD is caused by any condition that destroys nephrons. The three most common causes of ESRD are diabetes mellitus (34%), hypertension (25%), and chronic glomerulonephritis (16%). Other common causes include polycystic kidney disease, systemic lupus erythematosus, neoplasm, and acquired immunodeficiency syndrome (AIDS) nephropathy. Hereditary and environmental factors such as amyloidosis, congenital disease, hyperlipidemia, immunoglobulin A nephropathy, and silica exposure also contribute to the disease. [3]

1.2.3 Pathophysiology and complications

Human kidneys are two bean-shaped organs, one on each side of the spinal cord, and each one of them contains from one to two million nephrons. They represent about 0.5% of the total weight of the body and remarkably receive 20–25% of the total arterial blood pumped by the heart. [1]

Deterioration and destruction of functioning nephrons are the underlying pathologic processes of ESRD. The nephron includes the glomerulus, tubules and vasculature. Various diseases may affect different segments of the nephron at first, but the entire nephron can be eventually affected. Once lost, nephrons are not replaced. [3]

Nephron deterioration leads to successive laboratory and clinical stages. The first stage, called diminished renal reserve, is usually asymptomatic and is characterized by a mildly elevated creatinine level and a slight decline in GFR (10% to 20% change from normal). Progression leads to renal insufficiency, a term that is used when the GFR is mildly to moderately diminished, 20% to 50% of normal, and nitrogen products begin to accumulate in blood. In the third stage, called renal failure, the ability of the kidney to perform excretory, endocrine, and metabolic functions has deteriorated beyond compensatory mechanisms. This indicates that kidneys are unable to maintain normal homeostasis. The resultant clinical syndrome - caused by renal failure, retention of excretory products, and interference with endocrine and metabolic functions – is called uremia. Sequelae involve multiorgan systems including cardiovascular, hematologic, neuromuscular, endocrine, gastrointestinal, and dermatologic manifestations. [3, 4]

Among the most important complications of ESRD are the development of fluid overload, hypertension and risk of cardiac disease, which is caused by kidney incapacity to concentrate and filtrate the intake of sodium. Arterial hypertension is the most common; NaCl retention, fluid overload and inappropriately high rennin levels cause it. The cardiovascular system is affected by a tendency to develop congestive heart failure or pulmonary edema, or even both. [3]

There are also other complications as: azotemia, acidosis, electrolyte disturbances, anemia, hemorrhagic diatheses, committed host defense, renal osteodystrophy, and secondary hyperparathyroidism. [3]

Azotemia refers to the buildup of nonprotein nitrogen compounds, mainly urea, in the blood. [3]

Acidosis results from the combination of waste products, mostly ammonia retention. Patients with acidosis suffer of nausea, anorexia and fatigue, and due to adaptive mechanisms they tend to hyperventilate, which eventually may be fatal.[3]

Electrolyte disturbances, sodium depletion and hyperkalemia, occur as azotemia progresses, urine output falls and acid/base balance continues to deteriorate. [3]

Anemia is caused by decreased erythropoietin production by the kidney, inhibition of red blood cell production and hemolysis, bleeding episodes, and shortened red cell survival. [3]

Hemorrhagic diatheses, spontaneous or near spontaneous bleeding caused by a defect in clotting mechanisms (blood coagulation disorders) or another abnormality causing a structural flaw in the blood vessels (vascular hemostatic disorders), are common in patients with ESRD and are mainly attributed to abnormal platelet aggregation and adhesiveness, decreased platelet factor 3, impaired prothrombin consumption and defective platelet production. [3]

Host defense is compromised by nutritional deficiencies and changes in the production and function of white blood cells, therefore patients with these conditions are more susceptible to infection. [3]

Renal osteodystrophy includes a variety of bone disorders. The progression of osseous changes begins with osteomalacia (increased unmineralized bone matrix), it is followed by osteitis fibrosa (bone resorption, lytic lesions and marrow fibrosis), and finally, osteosclerosis in varying degrees (enhanced bone density). [3]

1.3 Clinical presentation

Clinical presentation of ESRD may be variable according to the severity of the disease and the context of the patient's overall physical status. [3]

1.3.1 Signs and symptoms

Patients with ESRD might be affected by conditions that will manifest as common signs and symptoms as anemia, hyperpigmentation, "uremic frost", multiorgan

system involvement, uremic syndrome, stomatitis, bleeding diatheses and cardiovascular related events. [3]

Patients generally appear ill and anemic and may develop nocturia. Anemia produces pallor of the skin and mucous membranes and contributes to the symptoms of lethargy, listlessness and dizziness. [3]

Hyperpigmentation of the skin is characterized by a brownish-yellow appearance caused by the kidney, pigments and might cause profound pruritus. [3]

“Uremic frost” is an occasional finding, refers to a whitish coating on the skin of the trunk and arms produced by residual urea crystals left when perspiration evaporates. [3]

Some of the organs involved are those from gastrointestinal system that provides signs such as anorexia, nausea, and vomiting, generalized gastroenteritis, and peptic ulcer disease. Cardiovascular system is also involved and presents signs such as shortness of breath, orthopnea and dyspnea on exertion, and peripheral edema. [3]

Uremic syndrome usually causes malnutrition and diarrhea, patients show mental slowness or depression and become psychotic in later stages, muscular hyperactivity, convulsion, which is a late finding and it could be directly correlated with the level of azotemia, stomatitis, manifested by oral ulceration and eventually candidiasis, parotitis and breath odor like urine. [3]

In what concerns bleeding diatheses, they can manifest as hemorrhagic episodes, particularly occult gastrointestinal bleeding, and it can also manifest as ecchymosis, petechiae, purpura, gingival or mucous membrane bleeding, and epistaxis. [3]

1.3.2 Laboratory findings

To monitor the progress of ESRD several tests are used such as: urinalysis, Blood Urea Nitrogen (BUN) Test, serum creatinine, creatinine clearance, electrolyte measurements, and protein electrophoresis. [3]

The most basic test of kidney function is urinalysis, with special emphasis on specific gravity and the presence of protein. [3]

Among the referred tests, three are used primarily to assess renal function: creatinine clearance, serum creatinine and GFR. Creatinine is a measure of muscle breakdown and filtration capacity of the nephron. It is proportionate to the glomerular filtration and tubular excretion rates and commonly is used as the index of clearance (creatinine clearance) in a 24-hour urine collection. [3]

The BUN is a common indicator of kidney function but is not as specific as creatinine level. [3]

1.4 Oral manifestations

The widespread of ESRD effects also implicate the involvement of stomatognatic apparatus, which can suffer a variety of oral signs: mucosal pallor (anemia), xerostomia, purpura, mucosal ulceration, white epithelial plaques, enamel hypoplasia and giant-cell lesions of the jaws. [3, 20]

1.4.1 Mucosal pallor

The pallor of the oral mucosa is related to anemia, which is one of most common oral changes affecting patients with ESRD. Another colour alteration is red-orange discoloration of the cheeks and mucosa, caused by pruritus and deposition of carotene-like pigments that occurs when renal filtration is decreased. [3]

1.4.2 Xerostomia

Xerostomia refers to a subjective sensation of dry mouth that may be associated or not with an impaired salivary function. A number of factors may play a role in the cause of xerostomia such as: developmental alterations, water or metabolite loss, iatrogenic origin, systemic diseases and local factors. [20-24] Additionally, a large number of commonly used medications, including drugs against psychiatric disorders and hypertension, have side effects of dry mouth, decreased saliva flow rate, and/or altered saliva composition. [21-23, 25]

The patient´s symptoms not always correspond to the clinical findings, in fact, some patients who complain of dry mouth may appear to have adequate salivary flow and oral moistness and conversely, some patients who clinically appear to have a dry mouth have no complaints. [20, 26]

In patients with reduced saliva flow rates all protective functions of saliva are negatively affected. [21] As a result, patients will have an increased caries experience, increased prevalence of oral candidiasis, due to reduction in the cleansing and antimicrobial activity normally provided by saliva, and patients may complain of difficulty with mastication and swallowing. [20, 21, 27-34]

Because of the increased potential for dental caries in patients with xerostomia, frequent dental visits are recommended. Office and daily home fluoride applications can be used to help prevent decay, and chlorhexidine mouth rinses minimize plaque buildup. [20]

1.4.3 Purpura

Purpura is a submucosal hemorrhage (Figure 1), which size is less than 2 cm. Purpura can arise from repeated or prolonged increased intrathoracic pressure and traumatic or non traumatic hemorrhage. When hemorrhage results from non traumatic causes, clinician should consider anticoagulant therapy, thrombocytopenia, disseminated intravascular coagulation (DIC) and a number of viral infections, especially infectious mononucleosis and measles, as possible causes. [20]

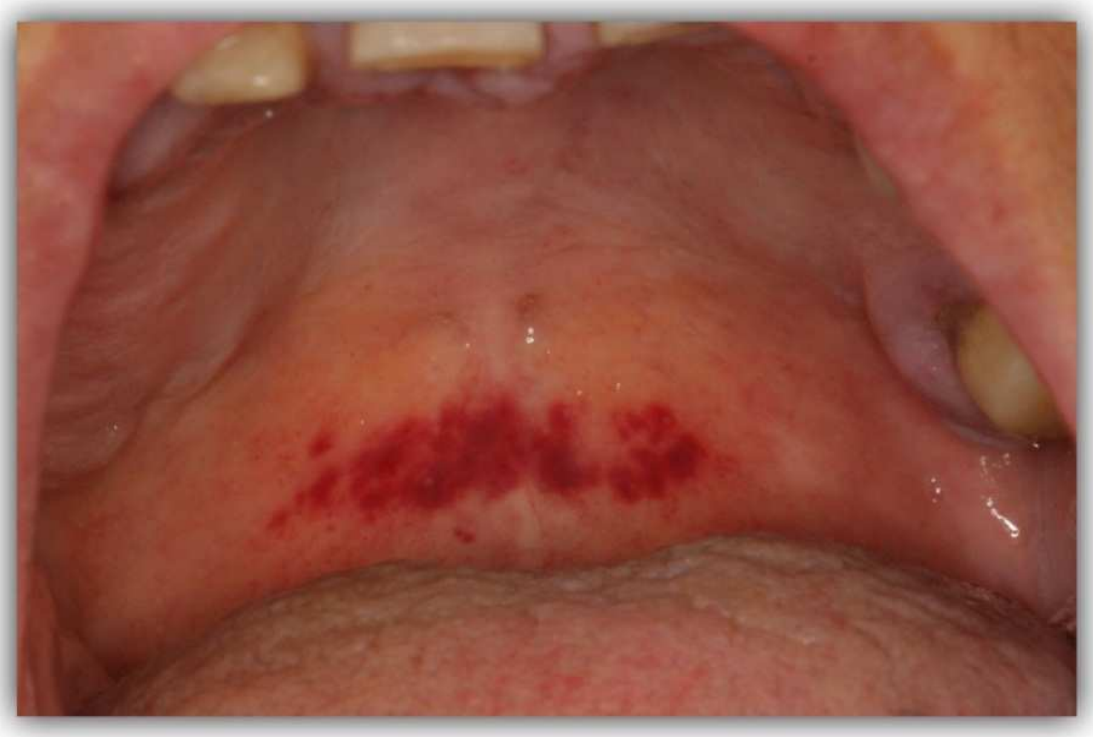


Figure 1. Submucosal hemorrhage.

1.4.4 Mucosal ulceration

Ulceration in the mouth is an open sore and it refers to a discontinuity of the oral mucosa that unable its normal functions. [20]

Ulceration may be an early manifestation, predating urinary abnormalities or renal failure. It is well recognized that new ulceration predicts increasing disease activity. Interestingly, there appears to be a link between oral ulceration and the development of lupus nephritis in patients with systemic lupus erythematosus, which could reflect an increased immunological organ injury. [35-37]

Mucosal ulceration might be an adverse effect of immunossupressive agents, such as mycophenolate mofetil, because it may cause inflammation and ulceration throughout the gut and it may give rise to painful oral ulceration, particularly in combination with sirolimus. [38] Henoch-Schonlein purpura can rarely present oral ulceration. [35]

1.4.5 White epithelial plaques

The presence of white epithelial plaques on the oral mucosa (Figure 2) might be caused by uremic stomatitis, a rare oral mucosal disorder associated with chronic renal failure, and it appears as adherent white plaques on any part of the oral mucosa, and may look like more common lesions such as lichen planus, chronic hyperplastic candidiasis, or even hairy leukoplakia. [35]

The cause of these often painful lesions is not known, but it is suggested the conversion of salivary urea by bacterial ureases to ammonia that causes a 'chemical burn'. [35, 39]

Uremic stomatitis in severe renal failure is early presented as red, burning mucosa covered with gray exudates and later as ulceration. [39]

Another white epithelial plaque is called "*uremic frost*" that is caused by urea crystal deposition. It is more common on the skin but may be seen on the oral mucosa too. [40]

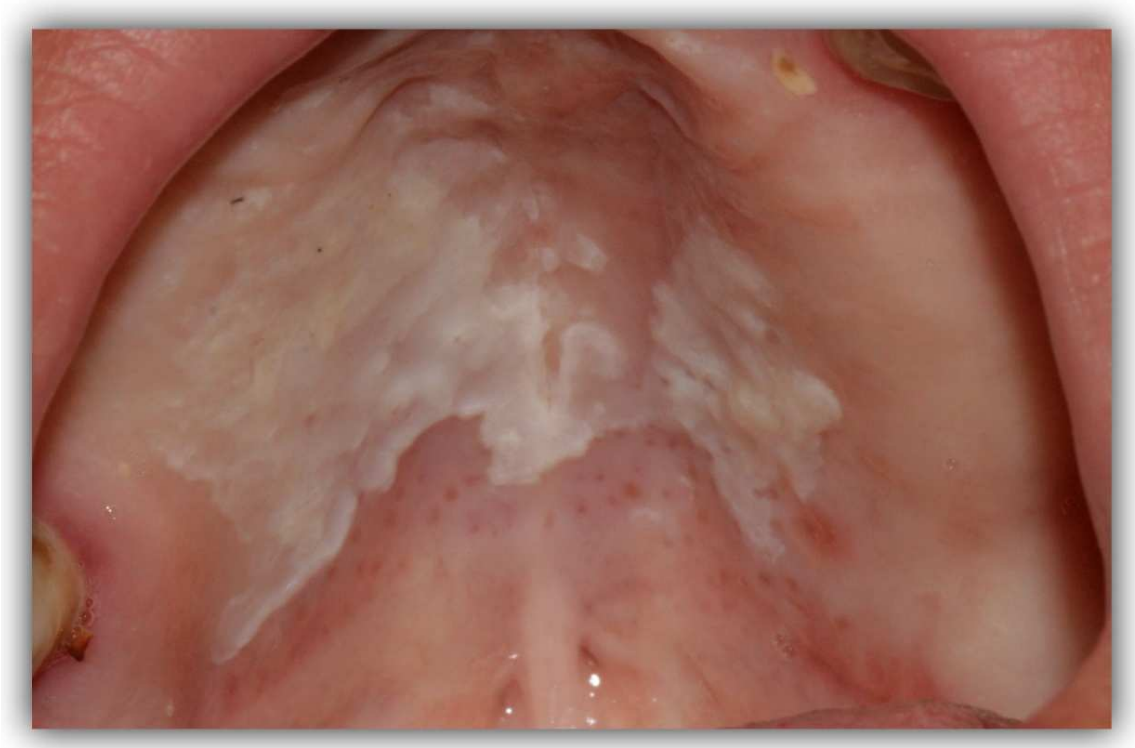


Figure 2. White epithelial plaque.

1.4.6 Enamel hypoplasia

Enamel hypoplasia is a defect in the teeth (Figure 3), in which the tooth enamel is hard but thin and it is deficient in amount. This is caused by defective enamel matrix formation with a deficiency in the cementing substance. [20]

Enamel hypoplasia has been documented in patients with ESRD whose disease began at an early age. In the developing dentition, it also have been reported red-brown discoloration and delayed or altered eruption. Tooth erosion could be a complication due patient persistent vomiting. Caries, however, is not a feature because salivary urea inhibits the metabolic end products of bacterial plaque and increases the buffering capacity of saliva, thus preventing a drop in pH sufficient to attain cariogenic levels. [3]

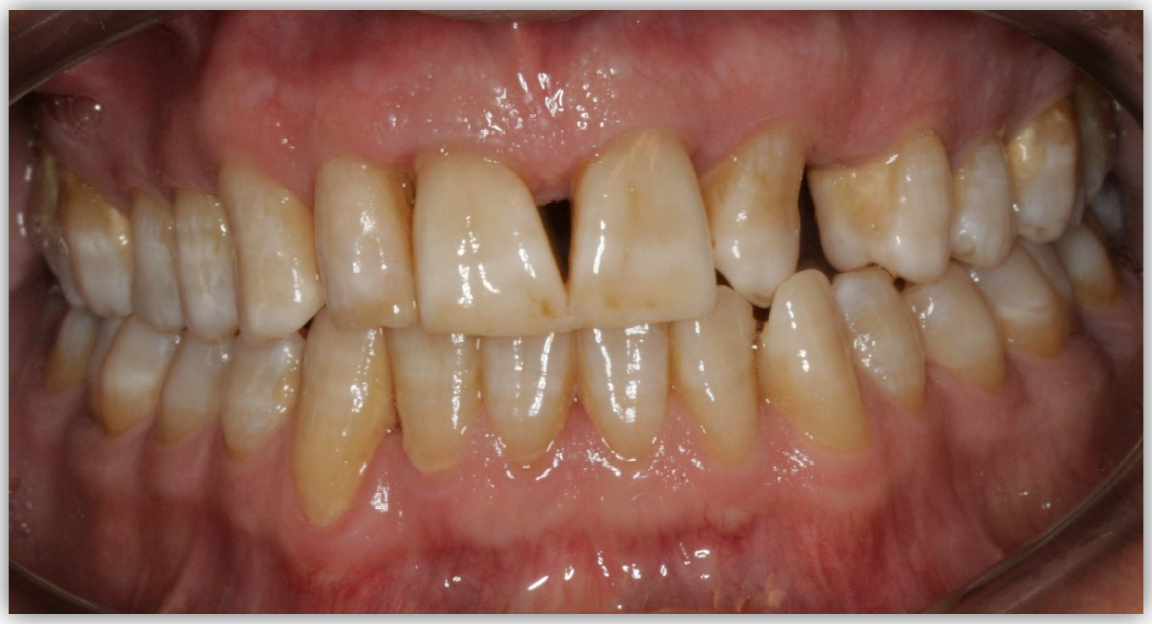


Figure 3. Enamel hypoplasia.

1.4.7 Giant-cell lesions of the jaws

Giant-cell lesions of the jaws are lytic bone lesions caused by secondary hyperparathyroidism. Other osseous findings include widened trabeculations, loss of cortication, calcification, calcified extraction sites ("socket sclerosis"), and metastatic calcifications within the skull. ESRD contribute to the development of

specific osseous changes of the jaws. The most classically described osseous change is the triad of loss of lamina dura, demineralized bone ("ground glass"), and localized radiolucent jaw lesions (central giant cell granulomas; "brown tumor"). [3]

2 Medical management of chronic kidney disease

On ESRD patients the following medical management can be used: conservative care, dialysis and kidney transplantation.

2.1 Conservative Care

Once the diagnosis of ESRD has been made, the goals of the treatment are to retard the progress of disease and to preserve the patient's quality of life. A conservative approach is the first step and may be adequate for prolonged periods. [3, 4]

Conservative care aims to slow the progression of kidney disease, avoiding nephrotoxic drugs or agents metabolized principally by it. To accomplish that is necessary to decrease the retention, fluids and electrolyte imbalances. This is possible by dietary modification-restricting protein and monitoring fluid, sodium, and potassium intake. Any treatable associated condition such as diabetes, hypertension, congestive heart failure, infection, volume depletion, urinary tract obstruction, secondary hyperparathyroidism, and hyperuricemia is corrected or controlled. In particular, secondary hyperparathyroidism is treated with low-phosphate binders (e.g., calcium carbonate), calcitriol, and other vitamin D preparations that decrease serum parathyroid hormone levels. Anemia is treated with use of recombinant human erythropoietin. [3]

2.2 Dialysis

Dialysis is a therapy able to extend life, it refers to a medical procedure that artificially filters the blood and it becomes necessary when the number of nephrons diminishes to the point that azotemia is unpreventable or uncontrollable. The procedure can be accomplished by peritoneal dialysis or by HD. [3, 4]

According to the Dialysis Outcomes and Practice Patterns Study (DOPPS) the mortality risk is relatively high in the early phase after initiation of dialysis, and among the causes of death there are those related to atherosclerotic heart disease and congestive heart failure. Additionally, the infection associated with catheter use for vascular access is also a cause of death. [4]

Most dialysis patients, 90%, receive HD. This treatment is performed every 2 or 3 days, depending on need, and it is usually required 3 to 4 hours for each session. Therefore HD consumes an enormous amount of the patient´s time what interferes with their daily routine. Between dialysis session, patients have a relatively normal life. [3]

Most patients receive HD through a permanent and surgically placed arteriovenous graft or fistula, usually placed in the forearm. Patients are "plugged in" to the HD machine at the fistula/graft site, and blood is passed through the machine, filtered, and returned to the patient. Heparin is usually administered during the procedure to prevent clotting. This procedure and the fact that HD only provides about 15% of normal renal function are both responsible for the development of complications such as: anemia, muscle tetany, over secretion of parathyroid hormone, risk of hepatitis B, C, and human immunodeficiency virus (HIV) infections, platelet destruction by mechanical trauma, development of osteomalacia. Other complication is the infection of the arteriovenous fistula that can result in septicemia, septic emboli, infective endarteritis and infective endocarditis. The risk of fistula infection from surgical procedures in dentistry is not precisely known but is considered low. [3]

Dental and oral status of patients receiving hemodialysis therapy

Patients receiving HD are at risk to oral complications and alterations in salivary composition and output. Additionally, vomiting and reduced oral (self) care could also negatively affect the oral health resulting in more caries, higher plaque and calculus indices, periodontitis and oral lesions. Symptoms like uremic odor, dry mouth, taste change, and tongue and mucosal pain are more frequent in these patients than among healthy patients. Similarly, some studies showed higher rates of oral pathology such as xerostomia, narrowing of the pulp chamber, enamel

abnormalities, and premature bone loss when compared with the general population (Figure 4). [3, 14, 35, 41]

HD patients are described in literature as having poor dental hygiene and the dental calculus may form faster, because of an imbalance in the calcium phosphate in serum [14, 35]. Moreover, plaque-related diseases can prove to be a source of active infection in these patients so it is recommended active oral treatment. [14, 41]

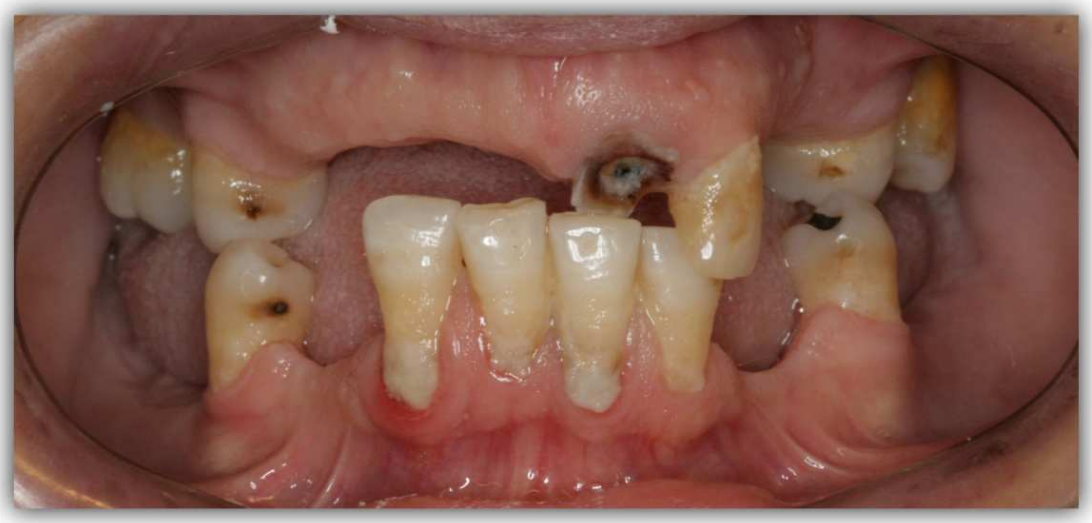


Figure 4. Premature bone loss and poor dental hygiene.

A taste disturbance is another complication of HD and might be caused by metabolic disturbances, the use of medication, a diminished number of taste buds and changes in salivary flow rate. [2]

Patients on hemodialysis might complain of an enlargement of the tongue and a metallic taste before each dialysis session, which are related to anemia and uremia. [14]

Literature reveals conflicting data about the effect of HD on oral health status. If for one hand it has been suggested that the caries activity is lower, calculus formation is enhanced and occur alterations in the periodontal tissues, on the other, no differences are found in parameters such as: gingival and plaque indices, level of periodontal attachment or caries when compared with the general population. [2, 14]

Some studies have demonstrated that periodontal health is poor in HD patients and it is correlated with markers of malnutrition and inflammation. One of the inflammatory markers described is C-reactive protein. Further than, it has been suggested that successful treatment of periodontitis leads to a decrease in inflammatory markers, with reduced C-reactive protein levels, interleukin-6, and tumor necrosis factor-alpha and partial restoration of endothelial dysfunction, and might reduce the high burden of cardiovascular disease in HD patients. [35]

There is considerable evidence that periodontitis-related microorganisms impair blood rheological parameters and thereby contribute significantly to accelerated systemic or local diseases that cause premature death in dialyzed patients. [42]

Xerostomia, or dry mouth, is a frequent and important complaint among HD patients, together with thirst it is correlated with interdialytic weight gains, which is a risk factor for cardiovascular morbidity. Patients that complain of dry mouth need regular dental review, because are predisposed to sialadenitis, caries, oral inflammation and infection. But there are other complaints, namely, taste alterations as bad taste, altered taste sensation or a metallic taste; and bad breath odor, experienced as ammonia-like odor. All of them are related to dry mouth and uremia itself. [35]

HD patients could have secondary hyperparathyroidism (HPT), and this could lead to oral complications such as: craniofacial bone alterations, loss of the lamina dura and pulp obliteration. Secondarily to HPT, although increasingly rare, could be advanced renal osteodystrophy that could involve the mandible with Brown tumors, enlargement of the skeletal bases, increased tooth mobility, and it is suggested that might be related to temporomandibular complaints. [2, 14, 35]

Premature bone loss in the jaw of HD patients also is well recognized, leading to mandibular and maxillary fractures. [35]

Long-term HD patients are susceptible to amyloidosis deposition, caused by 2-microglobulin. The tongue is a common site for it, particularly AA subtype, presenting as macroglossia, which may offer an accessible tissue for histological confirmation of the diagnosis. [20, 35]

Another important issue in dentistry is the dental management of HD patients that could be affected by important aspects related to the health status of these patients, namely: heparinisation before dialysis, possible hepatitis B or C carriage after chronic dialysis, permanent venous fistulae susceptible to infection, secondary hyperparathyroidism, oral lesions due to drugs and oral disease in chronic renal failure. [3, 20]

Before performing an oral surgery it should be assessed the hemostasis status of HD patients, because HD tends to aggravate bleeding tendencies through physical destruction of platelets and the use of heparin. It could be done ordering a battery of screening tests, including PFA-100 (laboratory analyzer termed platelet function assay), activated partial thromboplastin time (aPTT), and platelet count. Patients at higher risk are those with elevated laboratory values and history of gastrointestinal bleeding. [3]

To control the increased risk for bleeding in these patients, the dentist may use several means such as: provide dental treatment at the optimum time, usually on the day after HD because this treatment usually leaves the patients fatigued and with tendency to bleed; delay treatment until heparin is eliminated from the bloodstream (heparin lasts 3 to 6 hours after infusion); promote primary closure healing and when needed use pressure and hemostatic agents like thrombin, oxidized cellulose, desmopressin and tranexamic acid; performing major surgical procedures on the day after the end of the week of the HD treatment to provide additional time for clot retention before dialysis is resumed (for example, on a Monday/ Wednesday/ Friday weekly HD regimen, surgery performed on Saturday allows an additional day for clot stabilization before HD is resumed on Monday of the following week). The dentist may contact the nephrologist when necessary and request the elimination of the heparin dose during the first HD session after the surgical procedure (HD can be performed without heparin when hemostasis and clot retention are important); the dentist may also request the administration of protamine sulfate (usually done by a physician) if immediate care is needed because it will block the anticoagulant effects of heparin. [3]

The dentist should take into account that medication of HD patients, namely, anti-coagulant therapy, might influence the level of inflammation (gingivitis or periodontitis) and induce an increased bleeding on probing. Despite the fact that an improvement in oral hygiene measures reduces the amount of dental plaque and calculus, in these cases it might not result in a bleeding reduction. [2]

Furthermore, the dentist may consider antibiotic cover for dental surgical procedures, taking into account that HD patients´ permanent venous fistula for the dialysis lines is susceptible to infection; additionally drugs, including sedation, should not be given intravenously, because of the risk of damage to superficial veins which are patient´s lifelines. He also should be aware that HD removes some drugs from the circulating blood and this may shorten the duration of the effect of prescribed medications. Drugs removed during HD are those with low binding capacity to plasma proteins, on the contrary, drugs with high lipid affinity have high tissue binding and are not available for dialysis removal. Lastly, dosage amounts and intervals should be adjusted in accordance with advice from the patient's physician. [3, 20]

2.3 Kidney transplantation

Miller initiated kidney transplantation in 1965. Nowadays it is considered a routine therapy for the treatment of irreversible renal failures and an alternative to long-term dialysis. [3, 43, 44] Interestingly, less than half of patients on dialysis are considered for transplantation. [4]

It is widely accepted that kidney transplantation improves quality and length of life, and costs less than dialysis. However, patients have a higher risk of complications ranging from infection to rejection episodes and cancer, amongst others. [4, 45]

Patient's selection for kidney transplantation is an essential process but in the literature it is considered a problematic issue, one of the reasons is that available guidelines focus on patient evaluation and leave the selection to center´s discretion. [4]

Transplantation involves the surgical removal of a kidney from a donor and implantation of the kidney into a recipient. The donor may be a living first-degree relative or someone who has recently died (cadaver donor). [46]

The treatment of choice for ESRD is the transplantation with a living donor. Medical evaluation of the donor is aimed to detect medical abnormalities that would put them at risk for donor surgery or even to develop ESRD. [47, 48] The evaluation also should exclude diseases like infection or malignancy that may be transmitted to the recipient. Living donors should represent the healthiest members of society. [4]

2.3.1 Predictions of transplant outcomes

Traditionally, the predictors of outcomes considered on kidney transplantation were graft and patient survival, but nowadays two key outcomes are considered, namely, improved survival and quality of life when compared to dialysis. [4]

In recent years short-term patient and graft survival rates have improved significantly, however, no comparable improvement in long-term survival rates has occurred. This could be due to nephrotoxicity of some immunosuppressive agents like calcineurin inhibitors (CNIs), which contributes to chronic graft dysfunction; opportunistic infections, like emergent infections such as BK virus infections and infections generally assumed to have been relegated to the past such as cytomegalovirus (CMV) and urinary tract infections (UTI); and cardiovascular disease, more common than in the general population and it still is the leading cause of death with graft function. As a consequence, the K/DOQI workgroup on CKD, concluded that renal transplant recipients (RTR) should be considered to be in the highest risk group for cardiovascular events. [4, 45, 49, 50]

An important aspect, related to oral health, is that low-grade untreated dental infection in immunocompromised individuals has been suggested to contribute for morbidity and even predispose to transplant rejection. [35]

2.3.2 Chronic allograft nephropathy

Chronic allograft nephropathy (CAN) is a progressive renal dysfunction of the allograft that ultimately could lead to its loss. In literature CAN is considered a confusing term that lacks a proper definition. Traditionally, has been defined as a combination of histological features and transplant glomerulopathy. [51] Recently, CAN is considered a clinical diagnosis, which components are progressive deterioration in graft function, premature graft failure, and proteinuria, associated with histological changes of interstitial fibrosis, tubular loss and atrophy, and vascular and glomerular changes. It has two main causes: immunological, specifically the occurrence and severity of rejection episodes; and non-immunological, specifically hypertension, proteinuria, hyperlipidemia, and the nephrotoxic effects of immunosuppressive drugs. [4, 51]

Based up on circumstantial evidence, it is suggested that mediators of CAN are hyperfiltration, proteinuria, hypertension, cigarette smoking, hyperlipidemia, and reactive oxidative species (ROS) production. In fact, all of them are biologically plausible and have been reported individually to be related to future deterioration of transplant function and eventually graft loss. [4]

Graft loss has major human and economic sequelae, and it is associated with major increments in cardiovascular and all-cause mortality risks, reflecting the reverse of the published survival benefits of transplantation over dialysis, and ultimately can lead to death. Interestingly, CAN together with primary kidney disease, represent 50% of overall death for graft loss. [4, 52, 53]

Despite our improved understanding on mechanisms and natural history of CAN, none therapeutic or prevention strategies have been established. Therefore, the primary aim is to minimize exposure to risk factors for development of CAN and, when established, is to minimize exposure to risk factors for progression, including modification of immunosuppressive treatment. Lately, the focus has been on the differential effects of immunosuppressive agents with regard to nephrotoxicity, specifically, the reduction or withdrawal of CNI and their replacement with non-nephrotoxic agents, to limit the risk of allograft rejection. Nevertheless, there is reluctance to change immunosuppression in patients with stable graft function,

consequently, there are few data that supports the changing for prevent CAN, and the existing data demonstrates marginal benefits. [4]

2.3.3 Immunosuppressive agents

The commonly used immunosuppressants are the following: calcineurin inhibitors, namely, cyclosprorin (Sandimmun®) and tacrolimus (Prograf®); prednisone (Meticorten®); mycophenolate (Cellcept®); rapamycin inhibitors, such as, sirolimus (Rapamune®) and Everolimus (Certican®). [54-56]

2.3.3.1 Calcineurin inhibitors

Calcineurin inhibitors (CNIs), namely cyclosporine A (CsA) and tacrolimus (Tac), are immunossupressive agents that have been used since the early 1980s as a standard approach to reduce the number of allograft acute rejection episodes and enhance its short-term survival. [52]

Recently, has been reported that the greater benefit of CNIs appears to be a decrease in the number of acute rejections during the first months after transplantation, because in the long term chronic nephrotoxicity arises as a major toxic effect of CNIs and is associated with mild-to-moderate renal dysfunction. [53] So, the balance between preventing immunological allograft failures, avoiding over immunosuppression and managing nephrotoxicity is still an unresolved issue. [52, 53]

There has been a growing interest in the possibility of eliminating or reducing exposure to CNIs, however some documented experiences suggests that attempts to withdraw CNIs must be approached with caution and assessed in various types of patients. [57]

2.3.3.1.1 Cyclosporin A

Cyclosporin (Sandimmune®, Neoral®, Gengraf®) also termed with synonyms as CsA or Cyclosporin A, is used on prophylaxis of organ rejection in kidney, liver, and heart transplants; in dosages of 9±3 mg/Kg/day divided twice daily; and has been used with azathioprine and/or corticosteroids. It is a lipophilic cyclic

endecapeptide, isolated as an antifungal, from soil samples containing Cylindrocarpon lucidum BOOTH and Tolypocladium inflatum GAMS (fungi imperfecti). [43, 58]

Pharmacokinetic problems due to incomplete, unpredictable and inadequate absorption of original formulation of CsA led to the introduction of new microemulsion formulation of CsA (Neoral®), characterized by better absorption and a lower intra/inter-patient variability, allowing an improved long-term transplant outcomes. [43]

Cyclosporin A is absorbed in the gut and peak plasma concentration is reached after 3-4 hours, passively diffuses through cell membranes of the following cells: erythrocytes (50%), lymphocytes (5%) and lipoproteins (40%), and approximately 5% remains free in the plasma. Then, CsA is metabolised in the liver microsomes, and excreted after 6 hours mainly via the bile, through the faeces. [43]

The mechanism of action is the inhibition of production and release of interleukin II through its binding to immunophillin cyclophilin A and inhibiting the calcium dependent serine/threonine phosphatase calcineurin. At the same time, it inhibits selectively macrophage activation and IL-1 production, prevents the production of IL-1 receptors on T helper cells, inhibits IL-2 synthesis at low concentrations limiting clonal amplification of citotoxic T cell and it inhibits their ability to respond to IL-2 (probably blocking cell surface receptors). [43, 54, 58]

The clinical use of CsA is associated to well documented side effects, such as: nephrotoxicity, hepatotoxicity, diabetes, hypertension, biliary calculus disease, epilepsy, tremors, plasmocytoma, squamous cell carcinoma of the lips, kaposi's sarcoma, cephalalgy, sinusitis, conjunctivitis, hairy-leukoplakia, neurotoxicity, hirsutism, altered bone metabolism, gingival overgrowth and lingual fungiform papillae hypertrophy. [43]

2.3.3.1.2 Tacrolimus

Tacrolimus (Prograf®, Protopic®) also termed with the synonym FK506, is used as a potent immunossupressive drug in liver and kidney transplant recipients; in dosages of 0,2 mg/Kg/day in 2 divided doses given every 12 hours. [54, 58]

The mechanism of action is to suppress cellular immunity, namely, inhibits T-lymphocyte activation, possibly by binding to an intracellular protein, FKBP-12. The FK506–FKBP12 complex, binds to calcineurin, like the CsA–cyclophilin A complex, also resulting in inhibition of IL-2 transcription. Tacrolimus is 10 to 100 times more potent than CsA as an immunosuppressive agent. [54, 58]

Although Tac and CsA have been associated with similar side effects in respect to nephrotoxicity, neurotoxicity and the induction of a diabetic state, it has been suggested that Tac could be associated with less frequent oral health problems, namely gingival overgrowth. [59]

2.3.3.2 Prednisone

Prednisone (Meticorten®, Deltasone®, Liquid Pred®) also termed with synonyms deltacortisone and deltadehydrocortisone, is a systemic corticosteroid used in organ transplantation; in dosages of 5-60 mg/ day in divided doses given 1-4 times/day. [58]

Prednisone is a synthetic corticosteroid that is particularly effective as an immunosuppressant drug. The mechanism of action is to decrease inflammation by suppression of migration of polymorphonuclear leukocytes and reversal of increased capillary permeability, suppress the immune system by reducing activity and volume of the lymphatic system and suppress adrenal function at high doses. [58]

It has short-term side effects like all glucocorticoids and mineralocorticoids, such as high blood glucose levels, especially in patients with diabetes mellitus or on other medications that increase blood glucose (such as tacrolimus); and fluid retention, respectively. Additionally, it can also includes insomnia, euphoria and, rarely, mania (in particular, in those suffering from Bipolar disorders I and II). For

long-term side-effects it could cause Cushing's syndrome, truncal weight gain, osteoporosis, glaucoma and cataracts, type II diabetes mellitus, and depression upon dose reduction or cessation. [58]

2.3.3.3 Mycophenolate

Mycophenolate (CellCept®) is an immunosuppressant agent also termed with the synonym mycophenolate mofetil (MMF), which is used on prophylaxis of organ rejection concomitantly with cyclosporine and corticosteroids in patients receiving allogenic renal, cardiac or hepatica transplants; in dosages of 1 g twice daily. [58]

The mechanism of action is by inhibition of purine synthesis of human lymphocytes and proliferation of human lymphocytes. [58]

As with all immunosuppressants, a negative side effect is leaving the patient susceptible to infection. Among the most common side effects of MMF are high blood sugars and increased blood cholesterol levels. It is regularly noted other changes in blood chemistry such as hypomagnesemia, hypocalcemia, hyperkalemia, and an increase in BUN. [58]

2.3.3.4 Rapamycin inhibitors

Rapamycin (TOR) inhibitors, also known as mTOR inhibitors (mammalian target of rapamycin), are a new class of drugs, of which sirolimus (SRL) and everolimus (ERL) are examples, and are considered in CNI-free or CNI-sparing regimens for kidney transplant recipients, with the particular feature of offering renal protection because they are not nephrotoxic. [57, 60]

2.3.3.4.1 Sirolimus

Sirolimus (Rapamune®) is an immunosuppressant agent used on prophylaxis of organ rejection in patients receiving renal transplants, in combination with cyclosporine and corticosteroids; in dosages of 2 mg/day. It is an antifungal macrolide with potent antiproliferative activities that results in an immunosuppressive effect. [58]

The mechanism of action is by inhibition of T-lymphocyte activation and proliferation in response to antigenic and cytokine stimulation. More precisely, it inhibits cellular proliferation stimulated by growth factor–driven signal transduction in response to alloantigens, however, it doesn´t inhibit the production of interleukin that results from antigen-induced T-cell activation. Nevertheless, it binds to the intracellular immunophilin FKBP12 and blocks the activity of the mammalian target of rapamycin (mTOR) with potent inhibition of downstream signaling and progression from the G1 to the S phase of the cell cycle. As a result it reduces the incidence of acute rejection after renal transplantation, without appearing to cause significant inherent nephrotoxicity. However, when combined with CNI therapy, renal function often worsens as a result of potentiated nephrotoxicity. [52, 58]

Sirolimus (SRL) is structurally similar to Tac, rather than CsA, and also binds to FKBP12, but the sirolimus–FKBP12 complex binds to and inhibits the mammalian target of rapamycin (mTOR), rather than calcineurin, resulting in inhibition of cytokine-mediated lymphocyte signaling, rather than cytokine production. [54]

Among the more common side effects associated are hypertension, peripheral edema, chest pain, fever, headache pain, insomnia, hypercholesterolemia, hiperkalemia, hypokalemia, hypophosphatemia, hyperlipidemia, increased serum creatinine and symptoms related to gastrointestinal, genitourinary, hematologic, neuromuscular, skeletal and respiratory systems. [58]

2.3.3.4.2 Everolimus

Everolimus (Certican®, Afinitor®) also termed with the synonym RAD-001, it is an inhibitor with potent immunosuppressive and antiproliferative effects that is currently used as an immunosuppressant to prevent rejection of organ transplants, and it is also used in patients with renal cell cancer for its antitumor activity. [49, 61]

Recently, some attention has been paid to its highly effective action in preventing acute rejection in RTR, and additionally, much research has also been conducted on its use in a number of cancers. [49, 61]

Everolimus (ERL) is a derivative of SRL, it works similarly to SRL as an mTOR inhibitor, namely, it blocks growth factor-mediated proliferation of T cells, B cells and vascular smooth muscle cells and lately, like CNIs, it acts on cellular response. [52, 57]

2.3.4 Dental and oral health of renal transplant recipients

The dental and oral health of RTR will possibly reveal oral manifestations of the earlier CKD and oral complications of the transplantation. Among oral complications, stand out drug complications, particularly gingival overgrowth; oral infections, namely periodontitis, dental caries, candidiasis, viral infection and squamous cell carcinoma. It can also be noticed enlarged and friable "strawberry gums", pathognomonic of Wegener's granulomatosus which may be associated with alveolar resorption and increased tooth mobility, and the involvement of salivary glands. [35]

Additionally, the examination of the perioral regions may reveal some features such as: pseudo-ruggades of systemic sclerosis, presenting as decreased maximum oral aperture on mouth opening; may be observed oral mucosal telangiectasia, as may widened periodontal ligament spaces; keratoconjunctivits sicca; xerostomia; difficulties with swallowing; impaired wound healing. [35]

2.3.4.1 Drugs complications

A drug complication is a disease or injury that develops during the treatment of a preexisting disorder, and frequently alters its prognosis. [3]

One of the most frequent drug complications in mouth of RTR is gingival overgrowth (GO), and it is related to immunossupressive agents, namely, CsA. [35, 43, 62]

Immunosuppressants, and concurrent medical treatments, can lead to structural changes in the mouth, precipitating disease. Studies in the United Kingdom reveal that RTR are 2-3 times more likely to develop oral lesions when compared with the normal population, and often go less to the dentist. [35, 63]

2.3.4.1.1 Gingival overgrowth

Gingival overgrowth (GO), also known as medication-associated gingival enlargement, refers to an abnormal growth of the gingival tissues secondary to the use of a systemic medication. Characteristically, neither the epithelium nor the cells within the connective tissue exhibit either hyperplasia or hypertrophy. It is known that gingival collagen constantly undergoes physiologic remodeling, and the process must be tightly controlled to maintain a constant volume of the gingival tissues. In GO this balance is disrupted, and the increased gingival size is due to an increased amount of extracellular matrix, predominantly collagen, because there are interferences with its remodeling, namely, the degradation process. [20, 64, 65]

Gingival overgrowth is the main oral manifestation in transplant recipients who use calcineurin inhibitors. [66] In addition, GO may be exhibited by RTR who are taking CsA but also by patients with CKD medicated with phenyotoin or calcium channel blockers. In fact, the clinical presentation is very similar. In literature it is suggested that cyclosporine, phenytoin and nifedipine are all associated with calcium deregulation, which disrupts the normal collagen phagocytosis and remodeling process. If this is true, then the increased collagen does not occur from hyperplasia but from impaired collagen degradation and remodeling. [3, 20]

Gingival overgrowth incidence and severity are increased when CsA and nifedipine are prescribed together. However, other calcium channel antagonists (verapamil) used in association with CsA do not have this effect. [35]

Renal transplant recipients receiving Tac also can present GO, although most reported cases are associated with CsA. It is suggested that the use of Tac causes fewer oral side-effects than CsA, and further, replacing CsA with Tac can lead to a reduction or resolution of GO. [35, 43]

Several associations of GO with age, sex, drug dosage, duration of therapy or interval since transplantation have been hypothesized. The introduction of alternative immunosuppressant drugs have been suggested to allow better long-term transplant outcomes and a decrease in incidence of GO. [43] It should also be

taken into account the wide intra and inter-individual variability in the susceptibility of the CsA to induce GO. [43, 67]

Gingival overgrowth mainly affects the labial interdental papillae, although, in more extensive cases, may involve gingival margins, lingual, and palatal gingivae (Figure 5). It is often limited to adherent gingiva, but it may extend coronally and interfere with occlusion, mastication and language, without necessarily altering the underlying periodontium. Additionally, it is related to difficulty in maintaining a good oral hygiene resulting in an increased susceptibility to infections, caries, and periodontal diseases. Furthermore, these consequences may have a psychological impact and may in turn influence compliance with medical therapy. [20, 35, 43, 68, 69]

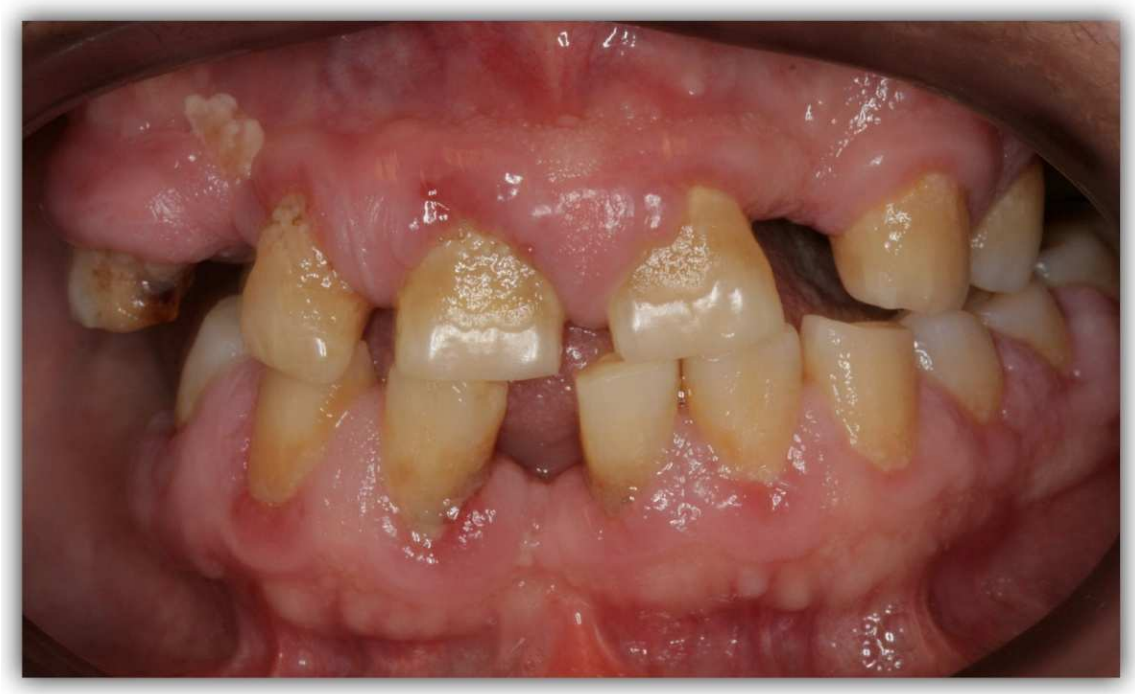


Figure 5. Gingival overgrowth.

Gingival overgrowth pathogenesis related to CsA is still uncertain and is probably multifactorial. It has been suggested that CsA is able to alter the metabolism of gingival fibroblasts increasing IL-6 secretion, and also, that gingival fibroblasts produce considerable quantity of IL-6, especially after stimulation with bacteria or other cytokines. [43, 70-72]

Increased levels of IL-6 have been reported in RTR, but the cellular origin of IL-6 in GO related to CsA is unknown. [43, 73, 74]

Gingival overgrowth, and its severity, is associated with risk factors such as: serum concentration and dosage of the drug, salivary concentration, time of administration, duration, age and sex, combinations of medications, genetic predisposition and oral hygiene. [43]

Different therapeutic approaches for GO management have been proposed. The use of specific oral hygiene programs, surgical intervention and/or alternative pharmacological therapy has been reported. [43]

Some authors consider that meticulous oral hygiene and frequent professional prophylaxis will help to reduce the effects of GO induced by CsA, however others report that additionally to oral hygiene, GO appears to be related significantly to the patient´s susceptibility. In fact, even with good oral hygiene some degree of gingival enlargement can be notice in susceptible individuals. Moreover, is generally accepted that rigorous oral hygiene often can limit the severity to clinically insignificant levels. [3, 20]

Chlorhexidine mouthwash may be beneficial. It seems reasonable to convert patients with intractable gum disease from CsA to Tac if they require a CNI, or avoid the class altogether. However, the nature of organ transplants could not permit an alternative therapy or dose reduction. [35, 43]

The need for surgical treatment needs to be carefully assessed. Surgery is normally performed for cosmetic/aesthetic requests before any functional need is showed. In cases where drug therapy must be continued for several years, psychosocial support needs to be considered with the purpose to reduce the frequency and extension of surgical interventions. [43]

2.3.4.2 Oral infections

Many systemic diseases like CKD can cause direct and indirect oral manifestations. The last one is the result of the host inflammatory or immune response modification and of host–parasite interaction balance disruption. This is crucial in

the pathogenesis of the two most prevalent oral infections – caries and periodontal diseases. [14]

The oral cavity may harbour different microbial microenvironments of heterogeneous compositions. More than 300 different bacterial species have been described together with other microorganisms, such as viruses, fungi and mycoplasmas. The subgingival biofilm is a complex bacterial community adhering to the root surface separated from the oral cavity by the soft tissue pocket wall. Under these conditions, many bacterial species with a high potential virulence, such as *Actinobacillus actinomycetemcomitans* and *Porphyromona gingivalis*, are able to colonize, grow and cause periodontal tissue damage. Systemic diseases, which may influence the microenvironment of periodontal pockets, could potentially affect the composition of this subgingival biofilm. In fact, patients with ESRD exhibit increased susceptibility to gingivitis and periodontal disease. [3, 14]

Fungal infection of the mouth is common. It is suggested that oral candidiasis will affect 20%-30% of RTR. [35]

2.3.4.2.1 Periodontitis

Periodontal diseases (PD) comprise a large group of disorders that affect the periodontium, which refers to specialized tissues that surround and support the teeth to the maxillary and mandibular bones, namely, gingival tissue, periodontal ligament, cementum and alveolar bone. PD has a multifactorial etiology and a prevalence of 20 to 50% on the worldwide population. [58] It includes gingivitis, chronic periodontitis and aggressive periodontitis. [75-78]

Gingivitis (Figure 6), the most common form of gingival inflammation, is a reversible inflammatory reaction of the dento-gingival tissues to bacterial plaque accumulation, which resolves soon after the dental bacterial biofilm is disrupted. [75]

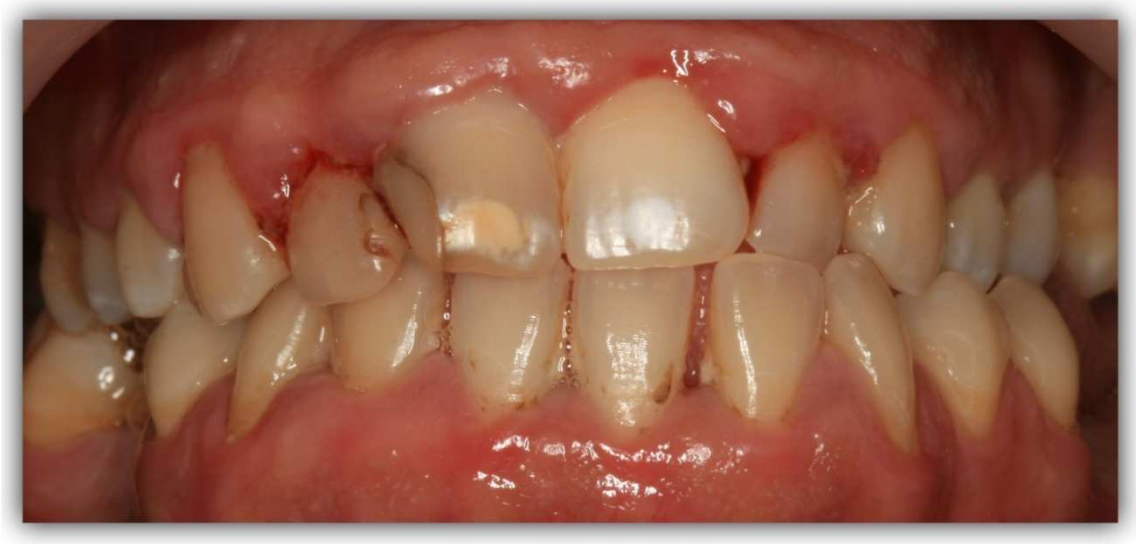


Figure 6. Gingivitis.

Periodontitis, in contrast to gingivitis, is a chronic inflammatory reaction involving not only superficial gingival tissues but also periodontal ligament and the alveolar bone. Usually is an asymptomatic condition with gingival bleeding and swelling representing the most common clinical signs. In an advanced form of periodontitis that refers to a progressive destruction of the dental supporting tissues signs can be found such as: gingival recession, drifting of the teeth, mobility and suppuration. And if it is left untreated, periodontitis may result in a progressive deepening of the gingival sulcus associated to alveolar bone destruction up to the apex of the tooth which eventually ends with its loss (tooth exfoliation), which is a major public health problem that affects a large number of older adults. [75-79]

Emerging evidence indicates that periodontitis is not a conventional infectious disease, but is an inflammatory disease triggered by host immune response to a constellation of periodontal biofilm-associated microorganisms. A dense mononuclear inflammatory infiltrate containing all cellular components acts between the infection and the targets of the disease (bone, connective tissue). Previous studies have shown that these inflammatory cells can infiltrate gingival tissues in an antigen-specific manner. [80, 81]

In what concerns chronic periodontitis (CP), the most common type of PD, is defined by the American Academy of Periodontology as an infectious disease that

results in inflammation of the tissues that support teeth, progressive loss of attachment and bone loss. This process leads to pocket formation around the tooth and/or gum recession. CP is initiated by the sub-gingival biofilm but its progression seems to be dependent of an abnormal host response to those organisms, generally accepted as the red complex, *Porphyromonas gingivalis, tanerella forsythia and Treponema dentícola.* [42, 82-84]

To classified CP, Armitage classification can be used, which describes low levels of CP as slight periodontitis, although other terms can also be used including mild, early and initial periodontitis. [85]

Aggressive periodontitis comprises a group of rare, often severe, rapidly progressive forms of periodontitis often characterized by an early age of clinical manifestation and a distinctive tendency for cases to aggregate in families. At the 1999 international classification workshop the following major common features characterized it: non-contributory medical history, rapid attachment loss and bone destruction and familial aggregation cases. [86]

Pathogenesis of periodontitis is described as a local inflammatory response elicited by the presence of subgingival pathogens. This is characterized by the formation of a local inflammatory infiltrate with exudation and migration of large number of leukocytes, involved in the first line of defense against bacterial pathogens, towards the affected area. Furthermore, this inflammatory response is exacerbated by the production of pro-inflammatory cytokines and prostaglandins. These are produced by a variety of cells involved in the response to the microbial invasion, such as monocytes/macrophages, neutrophils, lymphocytes, adipocytes and fibroblasts. The release of these substances into the bloodstream stimulates further recruitment of pro-inflammatory mediators and leukocytes at the local site. Cytokines such as IL-1 and IL-6 produced at the gingival sites might be dumped into the systemic circulation and stimulate a hepatic acute phase response to injury, and also stimulate haematopoiesis. All of this could cause damage to the structures that support the tooth, namely relocation of the junctional epithelium to the tooth root, destruction of the fibers of the gingival tissue, destruction of the periodontal ligament fibers and loss of alveolar bone support. Ultimately, these

damaged structures involve loss of bone height and eventually result in tooth loss. [42, 78, 84, 87]

Longitudinal studies established that the amount of alveolar bone loss or the number of teeth present at the baseline may be used to predict further progression of the disease, these variables are measures of the disease itself and express the level of susceptibility of a given subject to periodontal diseases. Although they may be excellent predictors for further disease progression, they can clearly not be considered as risk factors. [86]

A number of potential risk indicators that could be associated with PD have been suggested, such as increasing age, specific periodontal pathogens as *Porphyromonas gingivalis, Tannerella Forsythis* and *Fusobacterium nucleatum,* ethnic minorities, low socio-economic status, male gender and stress. Additionally, recent evidence suggests that common cardio-metabolic risk factors, including body weight, dyslipidemia and hypertension, are also associated with increased odds of prevalence of periodontitis. [78]

In what concerns cigarette smoking there is a considerable body of evidence demonstrating the association of periodontal destruction with it. The main effect of smoking is on the immune and inflammatory response and it is suggested that it results in the decrease of clinical signs, namely, gingival inflammation such as redness and bleeding. It is also suggested that smokers have a greater risk of exhibiting more periodontal attachment loss, larger number of deep periodontal pockets, higher mean probing pocket depth and more extensive and severe alveolar bone loss. Substantial epidemiological data indicate that smokers have fewer teeth, a higher prevalence of edentulism and a greater incidence of tooth loss than non-smokers. Another possible effect of smoking is on the microflora, however, its effect is inconclusive. [88, 89]

Periodontal diagnosis is predominantly based on clinical and radiographic measures. [90, 91]

In what concerns the measurement of periodontal health the accurate assessment is often performed by a trained examiner, using a periodontal probe. [90, 91]

Periodontal probes are used for this examination to help assess pocket depth and loss of attachment around each tooth and the presence of gingival bleeding. [90-92] This is then confirmed by radiographic assessment of the alveolar bone levels of all dentition. [90, 91]

The Florida Probe® introduced by Gibbs and co-workers [93] is an automated periodontal probe that has shown to be more reproducible than manual probing in a number of studies. At present this probe is considered the "golden standard" of the automated probes based on the extensive research on the validity of it. [91]

Clinical attachment level is defined as the distance from the cement-enamel junction to the tip of the probe. [86, 92] Attachment loss is measured (to the nearest mm) by simple probing by identifying the cemento–enamel junction and measuring the distance to the base of the pocket. [92] Probing depth is defined as the distance from the soft tissues margin to the tip of the probe. [86, 92] All teeth are examined. Third molars are excluded from analysis. [92]

Cemento-enamel junction is a fixed point that does not change; it refers to an anatomical border identified on a tooth that is the location where the enamel, which covers the anatomical crown of a tooth, and the cementum, which covers the anatomical root of a tooth, meet. This border is usually the location where the gingival tissue attaches to a healthy tooth by fibers called the gingival fibers. Because the bone level in health is approximately 2 mm apical to the CEJ, clinical attachment levels provide a reliable indication of the extent of bone support for a tooth. [86]

The recording of the probing depth is not considered a reliable indicator of the extent of bone support, because these measurements are made from the gingival margin which changes with tissue swelling, overgrowth, and recession. [91, 92] Additionally, in patients with untreated periodontal disease, remaining calculus, plaque and over contouring of restorations might also influence probing depth. [91]

In what concerns gingival bleeding during probing depth, bleeding on probing (BOP), it is a sign of inflammation. Bleeding can be visible immediately when a site is probed, or it may not be evident until about 10 seconds. BOP correlates with

gingival inflammation and is widely used in risk evaluation of periodontal disease progression. However, it has been shown that many sites with no progression of periodontal disease exhibited bleeding and thus, BOP has been considered a poor prognostic indicator for attachment loss in spite of its high degree of specificity. The relationship between BOP and periodontal progression is difficult to establish, as the results may be easily confounded by other factors such as smoking. It has been observed that smokers have less gingival bleeding when compared with non-smokers. [79, 94]

The periodontal status of each subject is assessed on the basis of the amount of CAL [92]. Two terms commonly used synonymously and abbreviated as CAL are clinical attachment level and clinical attachment loss. Clinical attachment loss is defined as the apical migration of the periodontal attachment from a reference point, which was supposed to be the normality. [92] Severity is characterized on the basis of the degree of attachment loss recorded in terms of the following codes: Health: Periodontal attachment loss 0 mm; Slight: Periodontal attachment loss 1 or 2 mm; Moderate: Periodontal attachment loss 3 or 4 mm; Severe: Periodontal attachment loss +5 mm or more. [92] Extent is characterized as 'Localized' = 30 % of sites involved, and 'Generalized' = ≥ 30% of sites involved. [92]

Nevertheless, clinical measurements provide us with an insensitive, retrospective analysis of what has already occurred, but allow us to diagnose disease based on its natural history. Measures of clinical attachment levels, by use of conventional probes, are only sufficiently sensitive indicators of periodontitis when as much as 20-30% of attachment has already been lost. Current technological improvements in probing measurements and radiographic assessment may increase sensitivity in this area. [78]

One of the challenges of periodontal practice is determining which patients with low levels of periodontal clinical attachment loss are most at risk for developing additional clinical attachment loss. This challenge extends to public health dentistry where population-based strategies are needed to reduce risk of periodontal infections that affect the dentition and can have an impact in systemic

health. CAL is considered a clinical predictor of periodontal disease progression. [85]

There have been signs that periodontitis may contribute to chronic systemic disease. [42] In fact, accumulating evidence suggests that the local inflammatory and/or infectious burden associated with periodontitis predispose to systemic diseases, namely, cardiovascular disease (CVD), CKD and diabetes. Indeed, periodontal status has been suggested to affect CVD traditional and newly risk factors, and it has been included in CKD multiple risk factors. [42, 75, 87, 95-98]

The explanation offered for this connection is that periodontal pathogens may circulate in the bloodstream and promote damage to blood vessel endothelium and atherosclerosis. It is plausible that such damage occurs not only in the endothelia of the heart and brain but also in kidney endothelium. [42]

Other explanation is that, similarly to acute bacterial infections, the persistent periodontal inflammatory state might have a repercussion on the total numbers of circulating neutrophils, because of increased bone marrow output or mobilization of the marginal granulocyte pool. Whether the increased number of leukocytes is mainly due to bacteremia or to excessive local production of inflammatory mediators remains unclear. [87]

Common risk factors for periodontitis and CVD have been described, as well as similar pathways have been proposed that include occurrence of transient bacteremia, elevation of inflammatory mediators in the systemic circulation in response to bacterial factors, endothelial and smooth muscle cell activation and molecular mimicry between bacterial and self-antigens. [87, 99] However, results might reflect possible confounders. [99]

In what concerns periodontitis and CKD, it is known that various chronic and acute infections are able to incite an inflammatory response in the kidney called glomerulonephritis, and periodontitis could be another one. Additionally, some researchers have shown the relation between periodontitis and kidney disease. [42, 100, 101]

The potential role of periodontal disease as a possible chronic source of infection and inflammation is supported by findings indicating its association with elevated serum levels of C-reactive protein (CRP) [99, 102, 103]. Recent studies have shown an association between high levels of CRP and interleukin-6 (IL-6) with periodontitis, an association that decreases after periodontal treatment [100, 104]. The association between periodontitis and systemic inflammatory response, determines the recently inclusion of periodontitis as a nontraditional risk factor for CKD. [99, 100, 102]

Despite all that has been published these associations are still not fully understood and are source of controversy, so further investigation is necessary.

2.3.4.2.2 Dental Caries

Dental caries is the most prevalent dental disease affecting human race although the prevalence of dental caries has significantly reduced; it is still a major problem. [105-107] The etiology and pathogenesis of dental caries are known to be multifactorial. [105-107]

Dental caries results in destruction of tooth structure by acid-forming bacteria that are found in dental plaque, which is an intraoral biofilm. The infection results in loss of tooth minerals, which begin on the outer surface and can progress through the dentin to the pulp, and this, can compromise the vitality of the tooth. [105, 106]

For RTR patients the immunosuppression status results in an increased risk of infectious complications and dental caries may represent an open door for bacteria. Therefore, dental caries treatment is indicated before patients start immunosuppression, and routine attendance to the dentist is advised for prevention. [108, 109]

An important factor for dental caries development and tooth demineralization/ remineralization rate is the secretion rate and quality of saliva. [106]

2.3.4.2.3 Oral Candidiasis

Oral candidiasis is a common fungal infection of the mouth that could affect 20% to 30% of allograft recipients. It refers to an opportunistic infection caused by

Candida albicans, a ubiquitous fungal organism that is part of the normal oral microbiota. [110]

In immunocompromised patients the presence of Candida albicans in the mouth can predispose to infections in other systems as esophageal, gastrointestinal, respiratory and urinary. [110] Candida albicans could trigger bloodstream and be an invasive form of infection with significant morbidity and higher risk for disseminated infection and eventually fatal. In RTR up to 80% of these infections were preceded by oral candidiasis or colonization. [110]

This infection may present as angular cheilitis, pseudomembranous or erythematous ulceration (Figure 7), or chronic atrophic infection. It also could be found in conjunction with occult esophageal infection and a history of odynophagia should alert physicians to the possibility of systemic candidiasis. [35, 110]

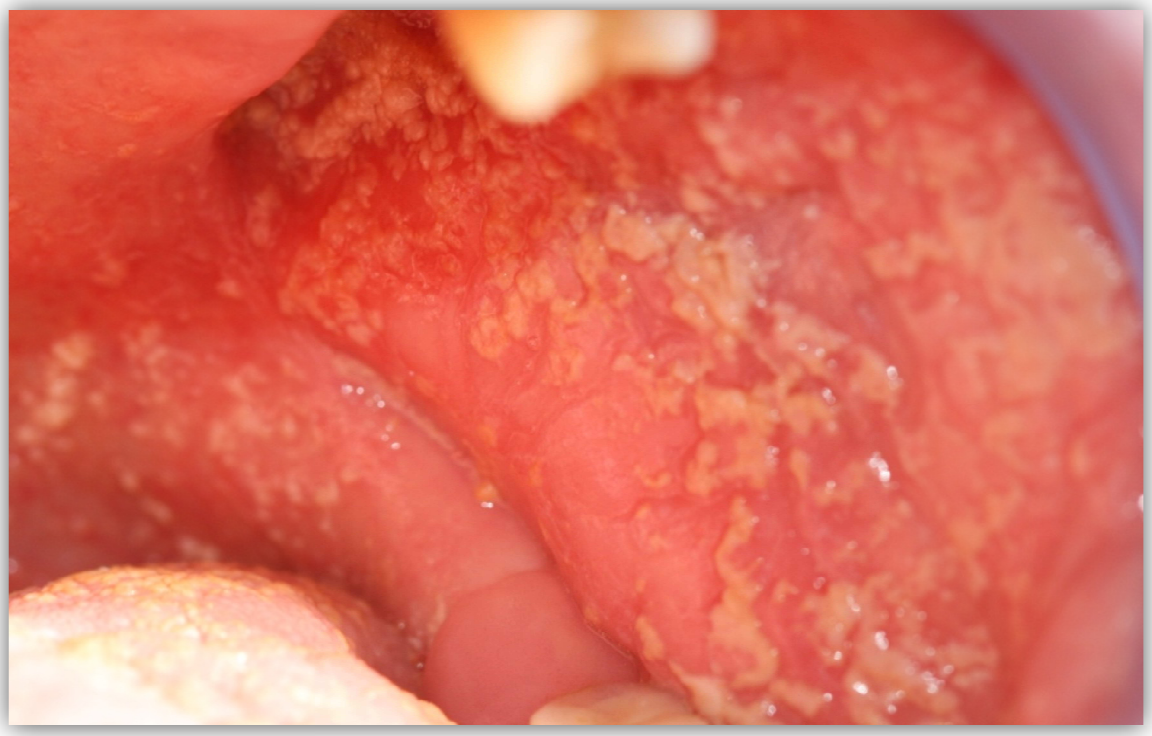


Figure 7. Pseudomembranous candidiasis.

Prevention with antifungal lozenges or solutions is simple and effective in the early post-transplant period (when corticosteroid doses may be highest). Treatment

depends on severity; lozenges may cure mild infections, but oral anti-fungals may be required, particularly if esophageal infection is suspected. [35]

2.3.4.2.4 Viral infection

In literature it has been reported that in RTR herpes simplex virus, cytomegalovirus and papilloma virus could cause viral infections in the mouth. Herpes simplex virus is a common and often troublesome infection in RTR, which frequency has been reduced by the use of antiherpetic agents, such as acyclovir. Not uncommonly Kaposi's sarcoma occurs and it could be caused by human herpes virus type 8. Cytomegalovirus infection often manifests with oral ulceration, usually in the context of tissue-invasive disease elsewhere. [35]

2.3.4.3 Squamous cell carcinoma

Squamous cell carcinoma is one of the most common cancers and usually arises from mutated ectodermal or endodermal cells lining body cavities. Therefore, it can develop in a large number of organs and tissues, including the skin, lips, mouth, esophagus, urinary bladder, prostate, lung, vagina and cervix, among others. Squamous cell carcinoma of the lip is considerably more common in RTR than in the normal population. [20, 35]

The cause of oral squamous cell carcinoma is multifactorial. No single causative agent or factor (carcinogen) has been clearly defined or accepted, but both extrinsic and intrinsic factors may be at work. Extrinsic factors include tobacco smoke, alcohol, syphilis and sunlight, which is only for lip vermilion cancers. [20]

Additionally, the papillary squamous cell carcinoma, which is a rare variant of the head and neck squamous cell carcinoma, is more frequent in patients with immunosuppression including those who have received a transplant. Established etiological factors can include tobacco smoking, heavy alcohol abuse and human papillomavirus infection. [111]

2.3.5 Dental management of renal transplant recipients

Renal transplant recipients may have special dental management needs, which may be similar to CKD patients, because they are affected by corticosteroid and other immunosuppressive treatments, hemorrhagic tendencies, anemia, impaired drug excretion, hypertension, hepatitis B or C carriage, underlying causes of ESRD namely, diabetes mellitus. Among the most important dental management needs are the monitorization of blood pressure, screening for bleeding disorders and anemia, and prompt treatment of oral infections or antibiotic prophylaxis. Any abnormal values should be discussed with the physician. [3, 20]

When surgical procedures are undertaken, meticulous attention to good surgical technique is necessary to decrease the risks of excessive bleeding and infection. Alterations in drug dosage may be needed according to the amount of kidney function that is present, and an important concern are the drugs that are excreted primarily by the kidney, or that are nephrotoxic, such as acyclovir, aminoglycosides, aspirin, nonsteroidal anti-inflammatory drugs, tetracycline and acetaminophen. [3]

Dental care should be considered a standard part of the management of RTR. Its goals are to restore the mouth to the healthiest condition possible and to eliminate possible sources of infection. Routine dental care should be a common practice, should begin before listing for transplantation and should consist in a detailed dental assessment, treatment of gum disease, caries and impacted molars. [3]

3 Saliva as a diagnostic tool for assessment of oral health

In healthy individuals saliva fluid covers the tissues in the mouth and is critical to the preservation and maintenance of oral health. [21, 112-115]

Characteristically, saliva is a very dilute fluid composed for more than 99% of water. [115, 116] Whole saliva is a mixture of the secretions from the parotid, submandibular, sublingual and minor salivary glands and gingival crevicular fluid. [117] Major salivary glands contribute for most of the secretion volume and

electrolyte content of saliva, whereas minor salivary glands contribute with little secretion volume and with most of the blood-group substances. Its normal composition is characterized by a variety of electrolytes, such as sodium, potassium, calcium, magnesium, bicarbonate and phosphates; immunoglobulins, proteins, enzymes, mucins; and nitrogenous products, like urea and ammonia. [115, 116]

Salivation is initiated by the salivary centers in the medulla oblongata, which receive afferent signals from the sensory terminals of the oral and nasal cavities and from the higher centers in the brain. [117] The secretion of saliva is regulated by the autonomic nervous system and its composition follows circadian rhythms. [117] Water and electrolyte secretion are mainly controlled by parasympathetic activity, whereas protein synthesis and exocytosis are mainly controlled by sympathetic activity. [118, 119]

Salivary function maintains oral health and creates an appropriated ecologic balance. It has 5 major functions: lubrification and protection, buffering action and clearance, maintenance of tooth integrity, antibacterial activity, taste and digestion. [21, 32, 113, 115, 120-122]

Interestingly, altered concentrations of various salivary electrolytes and ions may compromise several salivary functions related to remineralization, maintaining buffering capacity, taste mediatory role and digesting ability. [115, 116]

For the last ten years, researchers have shown increasing interest in using saliva to diagnose several diseases, to monitor therapeutic and illicit drugs and hormone levels, and to determine antibodies that protect the body from infectious processes. [114, 115]

Nowadays, saliva is already used to aid the diagnosis of dental diseases, in situations like: caries risk assessment, periodontal disease genotypes and markers identification, salivary gland diseases and dysfunction, and *Candida albicans* infections. [114, 115]

Some studies have demonstrated the usefulness of saliva and gingival crevicular fluid for the diagnosis of periodontitis. [123, 124] Whole saliva sampling is far easier,

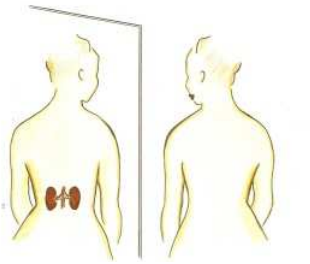
noninvasive and cheaper than gingival crevicular fluid collection. [123] In addition, collection of saliva is less costly and time-consuming. [123] Saliva contains many enzymes and some inflammatory markers. [123] These enzymes in serum have been routinely examined for the screening of systemic disease. [123] Therefore, no specific laboratory devices are necessary, and this approach is considered to be suitable for public health use. [123]

The value of saliva will certainly continue to increase because it is an easily collected, noninvasive source of information. Additionally, it might be used in clinically difficult situations, such as dealing with children, physically impaired and anxious patients, where collection of blood samples would be a complex task. [114, 115]

4 Aims

The study aims to evaluate the differences in the oral health status of renal transplant recipients administrated with Cyclosporin A, Tacrolimus or Everolimus, and compare it with patients on hemodialysis (pre-transplant) and healthy controls (living kidney donor). Furthermore, when observing alterations among groups, oral microbiota, salivary and serum biochemical parameters will be studied.

Chapter II – Material and Methods



1 Subjects

Renal transplant recipients receiving CsA, Tac or ERL as maintenance immunosuppressive regime and patients undergoing hemodialysis, 18 to 70 years old, followed-up in the post-transplant outpatient clinic of the Nephrology Department of "Hospital de S. João" were included in the study. All RTRs were taking corticosteroids (metilprednisone/prednisone) and an antimetabolite (mycophenolate mophetil). Living kidney donors were recruited as healthy control group. Patients with diabetes, active systemic infection and those who possess less than 8 of the ten most anterior teeth in the upper or lower dental arches were excluded. The study was approved by the Ethics Committee of "Hospital de S. João". All participants were recruited voluntarily after receiving detailed information on the study protocol and written informed consent was obtained in all cases. Demographic variables including age, gender, literacy, body mass index, time since transplant and pharmacological data were recorded for all subjects. A physician, blind to the transplantation status and immunosuppressive treatment, evaluated the oral health status in all patients through oral examination carried out by a single calibrated dentist. The calibration was carried out about 4 weeks before the start of the study in the Department of Oral Medicine and Oral Surgery, Faculty of Dentistry, University of Porto. Blood samples were collected after 12 hours overnight fasting on the morning of the oral examination. The serum levels of the

immunosuppressor were measured. Cyclosporin A and Tac blood levels were measured using using chemiluminescent microparticule immunoassay (Abbot Architect I System analyzer, Abbott, IL, USA) whereas ERL blood levels were measured using fluorescent polarization immunoassay (Seradyn Innfluor Certicann adapted to an Abbott TDx Flex analyser, Abbott, IL, USA). Glomerular filtration rate (GFR) was estimated using the Cockcroft-Gault formula [125]. An online application was built for patient's data collection and storage. This included two independent input forms, one intended for the general characterization of clinical and demographic data and the other for the oral evaluation status. Data were stored in a common database, aggregated and then exported for further statistical analyses.

2 Oral evaluation

Oral hygiene habits were evaluated by inquiring the participants about the daily tooth brushing habits and how often the toothbrush was changed yearly. Oral symptoms were evaluated by asking the participants if they had the feeling of a dry mouth during the day and if they had the feeling of a bad breath during the day.

Oral hygiene was assessed using the Visible Plaque Index (VPI) and the Gingival Index. The VPI [126] was assessed in four sites of each tooth (mesio-buccal, mid-buccal, disto-buccal, mid-lingual); the percentage of the examined sites with visible plaque ranged from 0% to 100%. The gingival index [127] is scored as follows: 0 = normal gingiva; 1 = mild inflammation, slight change in color, slight edema, no bleeding on probing; 2 = moderate inflammation, redness, edema, and glazing, bleeding on probing; 3 = severe inflammation, marked redness and edema, ulceration, tendency to spontaneous bleeding.

For each participant the number of decayed (D), missing (M) and filled (F) teeth was recorded and the DMFT index was calculated following the World Health Organization recommendations. [126, 128] In addition, a full-mouth periodontal examination was performed for all the teeth present in the oral cavity, excluding the third molars, using a dental mirror, an explorer and the Florida Probe®

introduced by Gibbs and co-workers [93]. The clinical attachment loss (CAL) and the bleeding on probing (BOP) [127, 129] were recorded. CAL and BOP were assessed at 6 sites around each tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual). CAL was expressed as the distance in mm from the cemento-enamel junction to the bottom of the gingival pocket. [15] BOP was scored positive if a site bled after pocket probing within the time interval used for the buccal and lingual measurements of a quadrant. [76]

Gingival enlargement (GE) was measured per sextant using the Aas Index. [130]. We have followed a visual index, like Greenberg and co-workers [131], instead of an approach based on alginate impressions to avoid overburdening study participants who were receiving a full-mouth periodontal examination. Grade 0: no GE; grade I: slight or moderate GE; Grade II: marked GE; Grade III: severe GE; grade IV: very severe. Each sextant was graded according to the most severe site. A subject was classified as having GE when at least one interdental papilla with GE grade I was present in at least one sextant.

Additionally, whole saliva was collected in a quiet room over 5 minutes, between 8:00 to 12:00 AM to minimize the circadian rhythm effects, and at least 2h after eating, tooth brushing, mouth washing or smoking. Salivary secretion was stimulated with paraffin pellets (Ivoclar vivadent, NY, USA) and the participants were asked to spit into a sterile tube. The total amount collected over a 5-min period was registered, enabling the salivary flow rates (ml/min) to be calculated. The salivary pH was measured immediately after saliva collection using a pH indicator paper (5.0-8.0, Duotest, Germany).

3 Oral fungi characterization

Fungi quantification and identification was performed at FMDUP (classical microbiological methodology) and IPATIMUP (molecular biology methodology).

For fungi' isolation and quantification, a selective and differential culture medium was used: CHROMagar Candida™. The stimulated saliva samples were serially

diluted with 0.9% sterile NaCl solution until 10^{-2} and immediately plated in triplicate. Afterwards, plates were incubated aerobically for 48h at 37°C. Total number of colonies was counted, and the quantification results expressed in colony forming units (CFU) per ml of saliva (CFU/ml). The lower limit of detection was 10^2 CFU/ml.

Identification of *Candida albicans*, *C. tropicalis* and *C. krusei* was possible due to the specific colour of the colonies (green, metallic blue, pink, respectively). The other non-identified isolates were identified using a sequencing approach. Single colonies of non-identified yeast isolates were cultured on Sabouraud agar for 24h at 37ºC. A sodium hydroxide based method was used to extract DNA from yeasts following the protocol available at http://www.aspergillus.org.uk/indexhome.htm?secure/laboratory_protocols.

The identification of these microorganisms was based on the sequence analysis of 18s and internal transcribed spacer (ITS) regions employing a group of specific primers – EF3, EF4, fung5, ITS1-F, ITS4. [132, 133] The PCR reactions were performed in a Thermo-Hybaid-PX2 thermal cycler. Amplification products were visualized in a polyacrilamide gel and a silver-staining followed. Sequence analysis was used to identify gene fragments using a Genetic Analyzer ABI-Prism-3100 (Applied Biosystems). Genomic data obtained was compared with a database that comprises a large collection of yeast sequences of 18s and ITS regions from Genbank[1].

4 Saliva analysis

Biochemical parameters analysis was performed using an automatic analyzer, Pentra C200 (Horiba Medical, Montpellier).

For unstimulated saliva it was evaluated: alkaline phosphatase (ALP) U/L, aspartate aminotransferase (AST) U/l, L-lactate dehydrogenase (LDH) U/L, C-reactive protein (CRP) mg/l, LDL-Colesterol mg/dl, triglycerides mg/dl, uric acid mg/dl, urea mg/dl, creatinine mg/dl, total proteins mg/l, albumin mg/l, calcium

[1] (http://www.ncbi.nlm.nih.gov/genbank/).

mg/dl, Iron mg/l, magnesium mmol/l, phosphorus mg/dl, sodium ISE(U)-Na, potassium ISE(U)-K, clorum ISE(U)-Cl.

And for stimulated saliva it was evaluated: amylase, Immunoglobulin A CP, Immunoglobulin G CP, LDH ifcc, calcium mg/dl, Iron mg/l, magnesium mmol/l, phosphorus mg/dl, sodium ISE(U)-Na, potassium ISE (U)-K, chlorine ISE(U)-Cl.

In brief, sodium, potassium and chloride were evaluated by potentiometry using ion selective electrodes. UV using phosphomolybdate detected phosphate and calcium was determined by a photometric test, using ortho-cresolphthalein complexone. In addition, α-amylases were detected by an enzymatic photometric test, using as substract 4,6- ethylidene- (G7)-p-nitrophenyl-(G1)- α-D-maltoheptaoside (EPS-G7).

Several methods were used to evaluate the following parameters: ALP by the photometric method in according to IFCC; AST by UV method according to IFCC without pyridoxal phosphate; LDH by optimized method according to DGKC; LDH ifcc according to IFCC; CRP by turbidimetric method; LDL-cholesterol by homogenous method for direct determination of LDL; triglycerides by PAP enzymatic method; uric acid by Trinder method; urea by enzymatic test UV "Urease-GLDH"; creatinine by Jaffé method; total proteins by photometric method with Red Pyrogallol; albumin by colorimetric BCG (bromocresol green) method; iron by photometric test with Feren; magnesium with photometric test with Blue Xilidil; IgA and IgG by turbidimetric method.

5 Blood analysis

Blood collection was taken on the morning of the oral examination. Venous blood samples were taken from subjects after fasting for 12 h overnight.

The following blood parameters were consulted from medical records for all participants, namely 1) hemogram: hemoglobin (g/dl), hematocrit (%),leucocytes (%), neutrophils (%), eosinophils (%), basophils (%), lymphocytes (%), monocytes (%), platelets ($x10^9$/l); 2) general chemistry: total cholesterol (mg/dl), HDL

cholesterol (mg/dl), LDL cholesterol (mg/dl), triglycerides (mg/dl), glucose (mg/dl), urea (mg/dL), acid uric (mg/dl), creatinine (mg/dl), calcium (mEq/l), sodium (mEq/l), potassium (mEq/l), chloride (mEq/l) inorganic phosphorus (mg/dl), iron (ug/dl), magnesium (mmopl/l), transferrin (mg/dl), ferritin ng/ml, iron /transferrin x 70.9 (%); 3) proteins: total proteins (mg/l), C-reactive protein (mg/ml), albumin (mg/l), aspartate aminotransferase (U/l), alanine aminotransferase (U/l), alkaline phosphatase (U/l); 4) immunoglobulins: immunoglobulin A (mg/dl), immunoglobulin G (mg/dl), immunoglobulin E (mg/dl); 5) complement: C3c (mg/dl), C4 (mg/dl); 4) endocrinology: vitamin D (pg/ml), parathyroid hormone (PTH-I) (pg/ml).

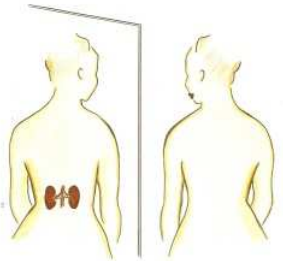
6 Ratio of biochemical parameters in saliva and blood

Ratio was calculated for each biochemical parameter studied. This value represents the quotient between the median of a parameter in saliva and the median of the same parameter in serum.

7 Statistical analysis

The categorical variables were described through absolute and relative frequencies (%) and analyzed by Chi-square independence test. Continuous variables were described using mean ± standard deviation (SD) and analyzed by one-way ANOVA when following a normal distribution. When continuous variables did not follow a normal distribution they were described using median, mean± standard deviation and analyzed by Kruskall-Wallis or Mann-Whitney test. A level of 0.05 was considered significant. The analyses were performed using the statistical analysis program SPSS® v.17.0 (Statistical Package for Social Sciences).

Chapter III – Results



1 Subjects

A total of 88 RTRs receiving CsA (n=29), Tac (n=36) or ERL (n=23), 23 patients undergoing HD (n=23) and 13 LKDs were included in the study. The CsA, Tac and ERL daily dosages and corresponding blood levels are given in Table 1. The pre-transplant dialysis vintage did not differ among the three groups of RTRs (Table 1). The median time on hemodialysis (min-max) was 53(24-204) months.

Statistically significant differences were observed in the time after transplant among the three groups of RTRs (Table 1). Through multiple comparisons tests are observed differences and reveal that RTRs receiving ERL presents median values significantly higher when compared with the other two RTRs groups. This result is illustrated in the graph of Figure 8.

Both serum creatinine levels and GFR were similar between RTRs receiving CsA, Tac and ERL (Table 1). As expected, renal function was well preserved in LKD group [serum creatinine 0.8 mg/dl (0.5-1.5); GFR 84 ml/min (68-207)].

Table 1. Daily dosage and corresponding blood levels of CsA, Tac and ERL. Renal history among RTRs receiving CsA, Tac and ERL.

	CsA (n=29)	**Tac** (n=36)	**ERL** (n=23)	**p**
Immunossupressor therapy				
Dosage (mg/day)	200 (100-400)	4.8 (1.5-12.0)	2.0 (0.5-3.0)	-
Blood level (ng/ml)	143.99±62.09	8.21±2.39	7.27±1.85	-
Renal history				
Time after transplant (months)	11 (0-201)	17 (0-152)	119 (20-216)	**<0.001**†
Glomerular filtration rate (mL/min)	52 (23-74)	54 (11-128)	50 (21-110)	0.641†
Pre-transplant dialysis vintage (months)	45 (8-174)	36 (3-87)	26 (4-62)	0.111†

Values are presented as median (min-max) except blood levels presented as mean ± standard deviation. Testing of group differences by †Kruskall-Wallis test.

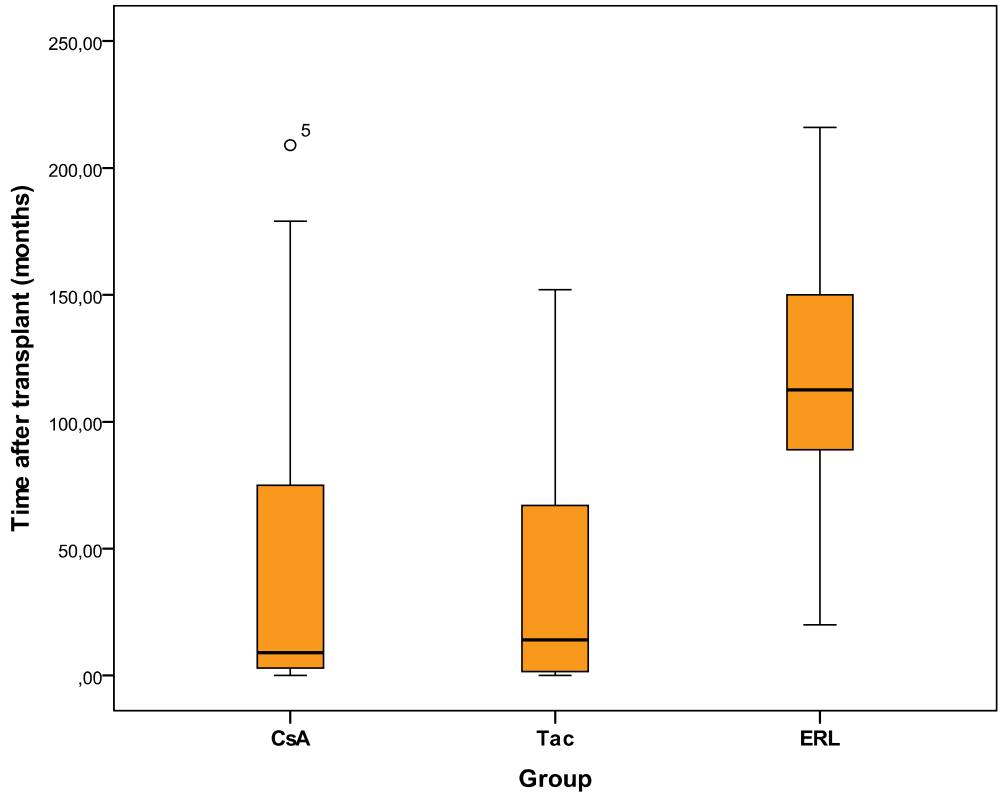


Figure 8. Distribution of time after transplant by group.

The RTRs receiving Tac were younger than RTRs receiving CsA and ERL, or HD and LKD groups (Table 2). This result is illustrated in the graph of Figure 9.

The prevalence of female gender was higher in LKD group in comparison with the others studied groups (Table 2). This result is illustrated in the graph of Figure 10.

No significant differences were observed in body mass index, systolic blood pressure, diastolic blood pressure and heart rate, among all the studied groups (Table 2). The smoking habits, both current and past did not differ significantly among all the studied groups (Table 2).

Table 2. Age, gender, literacy, body mass index, blood pressure, heart rate and smoking habits among RTRs receiving CsA, Tac and ERL, HD and LKDs groups.

	CsA (n=29)	**Tac** (n=36)	**ERL** (n=23)	**HD** (n=23)	**LKD** (n=13)	**p**
Age (years)	55(8)	39(9)	50(11)	52(8)	44(9)	**<0.001**#
Gender						**0.002***
Male	15 (51.7)	19 (52.8)	16 (72.7)	17 (73.9)	1 (7.7)	
Female	14 (48.3)	17 (47.2)	6 (27.3)	6 (26.1)	12 (92.3)	
Literacy						
Sixth grade	11 (78.6)	10 (66.7)	6 (66.7)	4 (100)	3 (75)	-
Higher than sixth grade	3 (21)	5 (33)	3 (33)	0	1 (25)	-
Body mass index	24(2)	24(4)	25(4)	26(1)	23(2)	0.357#
Blood pressure						
Systolic	137(12)	134(14)	133(15)	131(15)	123(20)	0.428#
Diastolic	81(9)	80(11)	77(13)	80(9)	68(11)	0.261#
Heart rate	75(15)	80(12)	75(11)	82(15)	63(2)	0.160#
Smoking habits						
Current smoking	5(17)	16(44)	6(29)	8(35)	4(31)	0.225*
Past smoking	15 (52)	19(53)	11(52)	15(65)	6(46)	0.810*

Values are presented as number (%) except age and body mass index presented as mean ± standard deviation. Testing of group differences by # one-way ANOVA or *chi-square test.

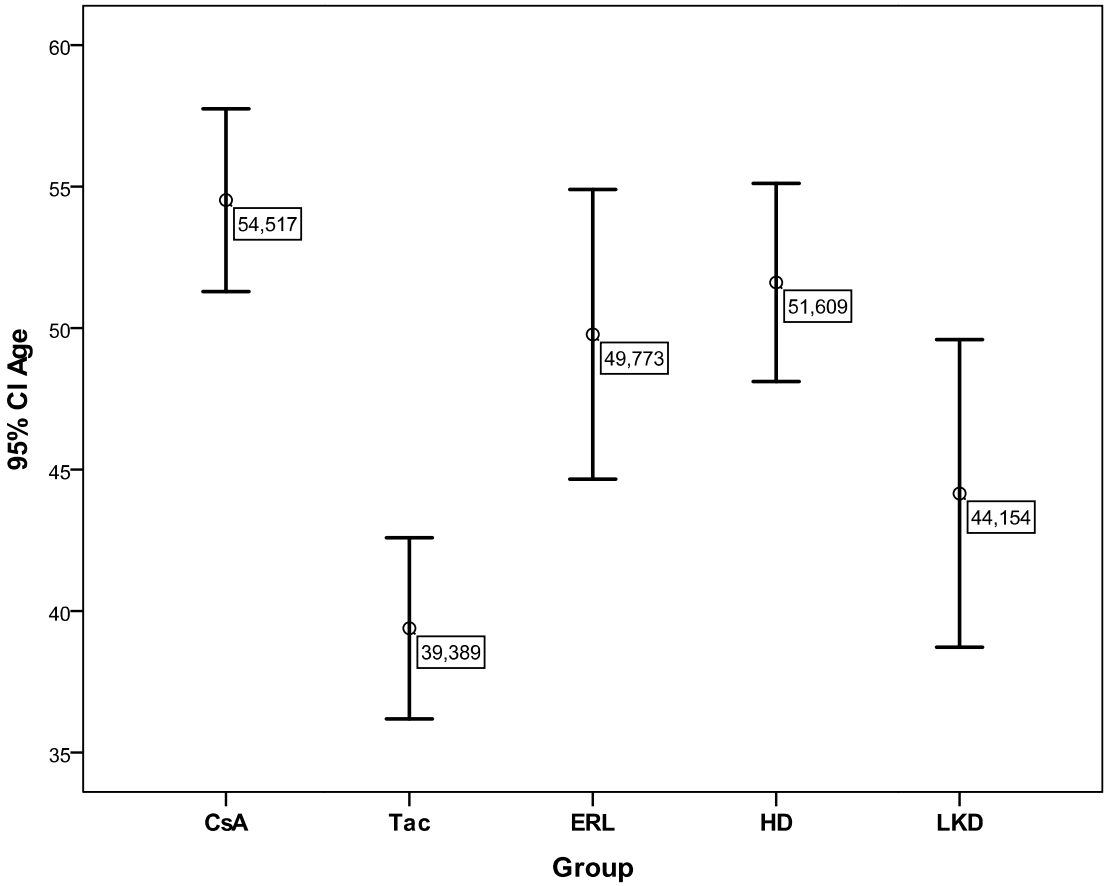


Figure 9. Distribution of age by group.

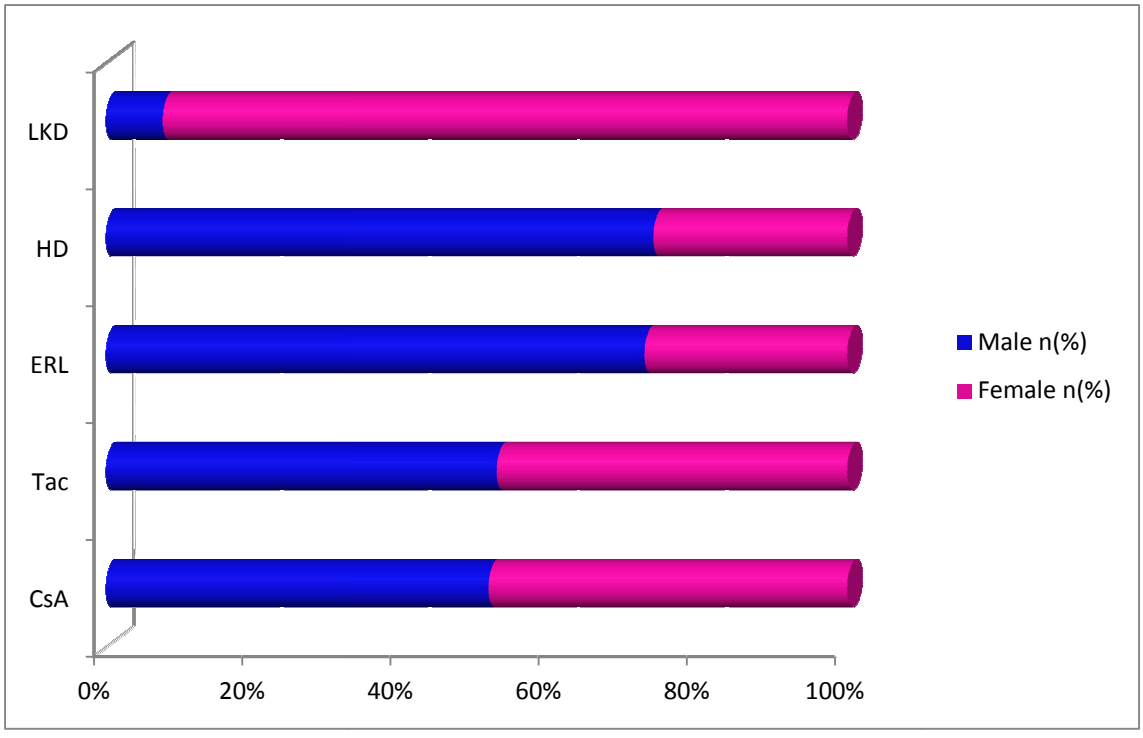


Figure 10. Distribution of gender by group.

2 Oral health status

The oral health evaluation data among RTRs, HD patients and LKDs is given in Table 3. No significant differences were observed in oral hygiene habits and oral symptoms among the three groups (Table 3). In addition, both visible plaque index and DMFT index did not differ among the three groups (Table 3). No differences were found for bleeding on probing among the three groups (Table 3). Concerning clinical attachment level, positive cases for gingival enlargement were excluded, and no differences were found among the three groups (Table 3).

The results of gingival enlargement (Figure 11) failed to detect statistically sufficient evidence on the association with the groups (($\chi 2=2.588^{a2}$, df=2, $p>0.05$). Nevertheless, the graph of Figure 12 illustrates a GE trend to be associated to RTRs group. Regarding GI, no statistical analysis could be performed due to the reduced number of cases in gingival index scores. The graph of Figure 13 illustrates a similar distribution of GI scores across groups.

Unstimulated and stimulated saliva pH did not differ among the studied groups (Table 3). Statistically significant differences were observed in both unstimulated and stimulated flow rate among the three groups (Table 3). Through multiple comparisons tests are observed differences in both unstimulated and stimulated flow rate. These differences reveal that HD patient's present median values significantly lower when compared with RTRs and LKDs groups. These results are illustrated in the graphs of Figures 14 and 15.

No statistically significant differences were observed in the distribution of unstimulated (Kruskall-Wallis test, Z=1.198, p=0.113) and stimulated (Kruskall-Wallis test, Z=1.246, p=0.090) saliva flow rate in the individuals with or without the feeling of a dry mouth, as can be illustrated by the graphs in Figure 16. Even though the individuals with the feeling of a dry mouth presented a median values of unstimulated and stimulated saliva flow rate lower than those who do not have this feeling.

[2] a. 2 cells (33,3%) have expected count less than 5. The minimum expected count is 1.11.

Table 3. Oral hygiene habits, oral symptoms, teeth evaluation, periodontal evaluation and saliva flow rate and pH evaluation in RTRs, HD and LKDs groups.

	RTR (n=88)	HD (n=23)	LKD (n=13)	p
Oral hygiene habits				
Daily tooth brushing <2 times per day	28(37.3)	11(57.9)	5(38.5)	0.260*
Change toothbrush <4 times per year	33(37.9)	3(13.0)	5(38.5)	0.255*
Oral symptoms				
Dry mouth	44(59.5)	13(68.4)	6(46.2)	0.452*
Bad breath	45(60)	14 (73.7)	10(76.9)	0.326*
Teeth evaluation				
Visible Plaque index	94(87.4±18.7)	100(90.5±15.1)	85(83.2±13.7)	0.138†
Decayed median	1(2.5±3.6)	1(2±2.2)	1(2.2±2.3)	0.792†
Missing median	0(1.5±2.6)	0(1.5±2.9)	1(2.6±3.3)	0.248†
Filled	6(7.3±6)	7(8±7.7)	11(11.6±8.2)	0.184†
DMFT index	11(11.1±7)	10(11.5±8.6)	18(16.4±8)	0.086†
Periodontal Evaluation				
Bleeding on probing	6 (14.1±19.6)	4 (12.3±21.9)	5.5 (9.9±10.5)	0.753†
Clinical attachment level	2.9 (2.9±0.7)	3.1 (3.5±1.3)	2.6 (2.6±0.7)	0.102†
Salivary Evaluation				
Unstimulated saliva flow rate (ml/min)	0.4 (0.4±0.29)	0.26 (0.28±0.18)	0.26 (0.35±0.29)	**0.036**†
Unstimulated saliva pH	7.4 (7.14±0.51)	7.4 (7.24±0.69)	6.8 (6.89±0.46)	0.120†
Stimulated saliva flow rate (ml/min)	1.36 (1.55±0.88)	0.88 (1.02±0.49)	1.16 (1.42±0.81)	**0.024**†
Stimulated saliva pH	8.0 (7.75±0.4)	8.0 (7.73±0.43)	7.7 (7.65±0.36)	0.435†

Values are presented as n (%) for categorical variables or median (mean±standard deviation). †Testing of group differences by Kruskall-Wallis test. *Testing of group differences by chi-square test.

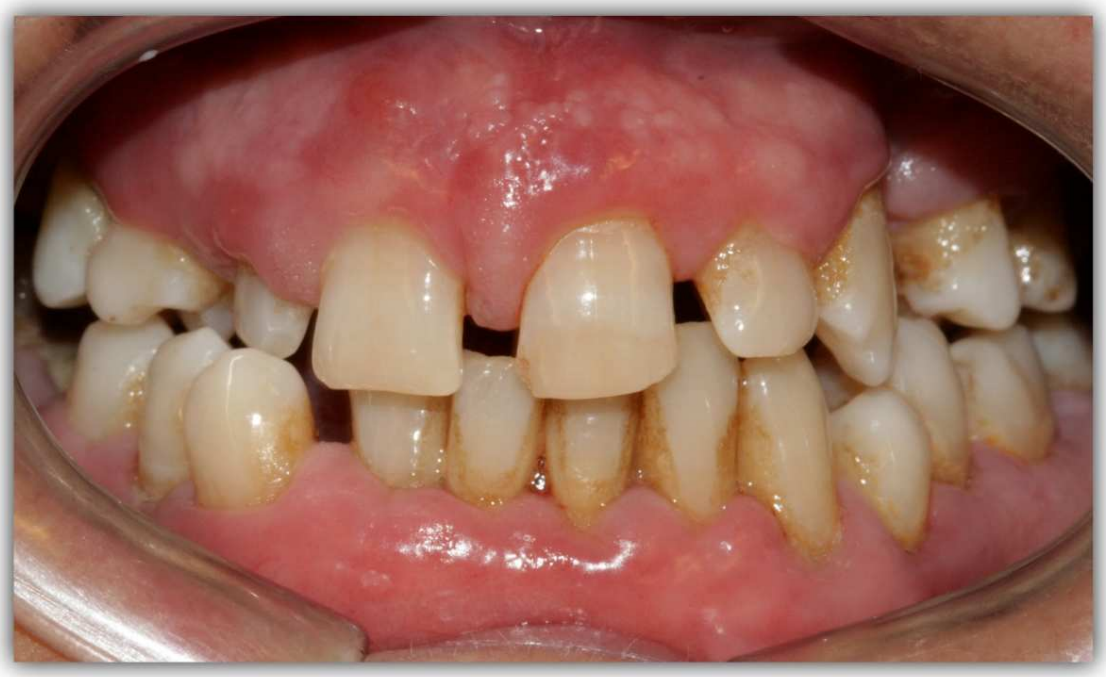
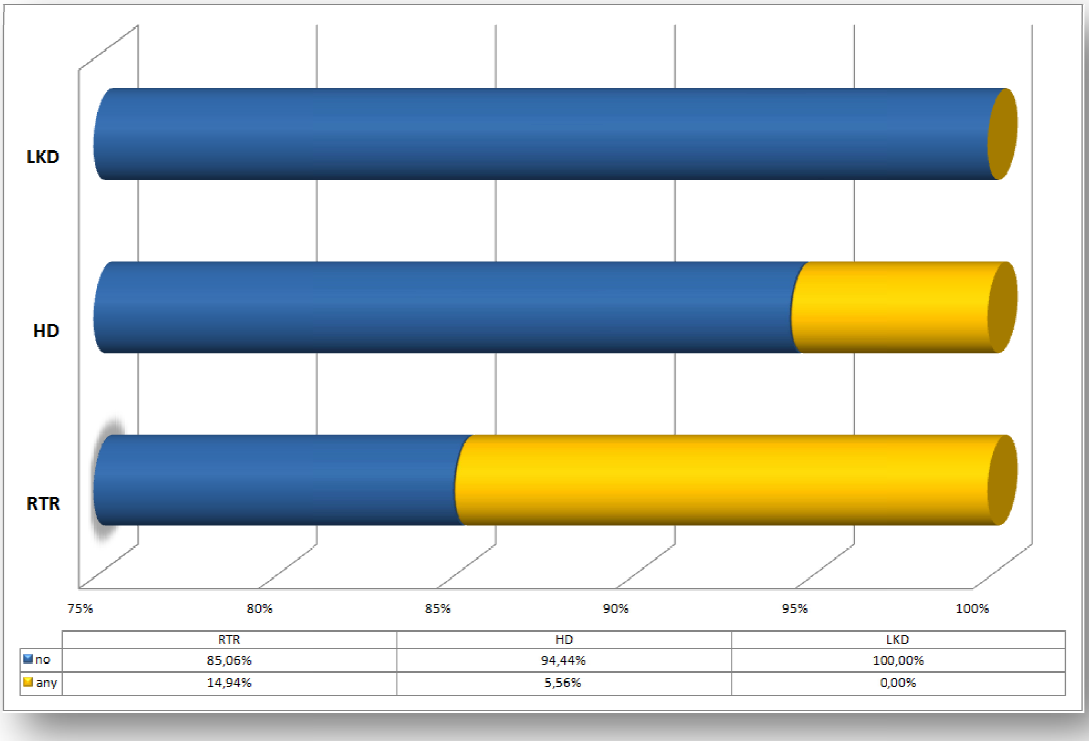
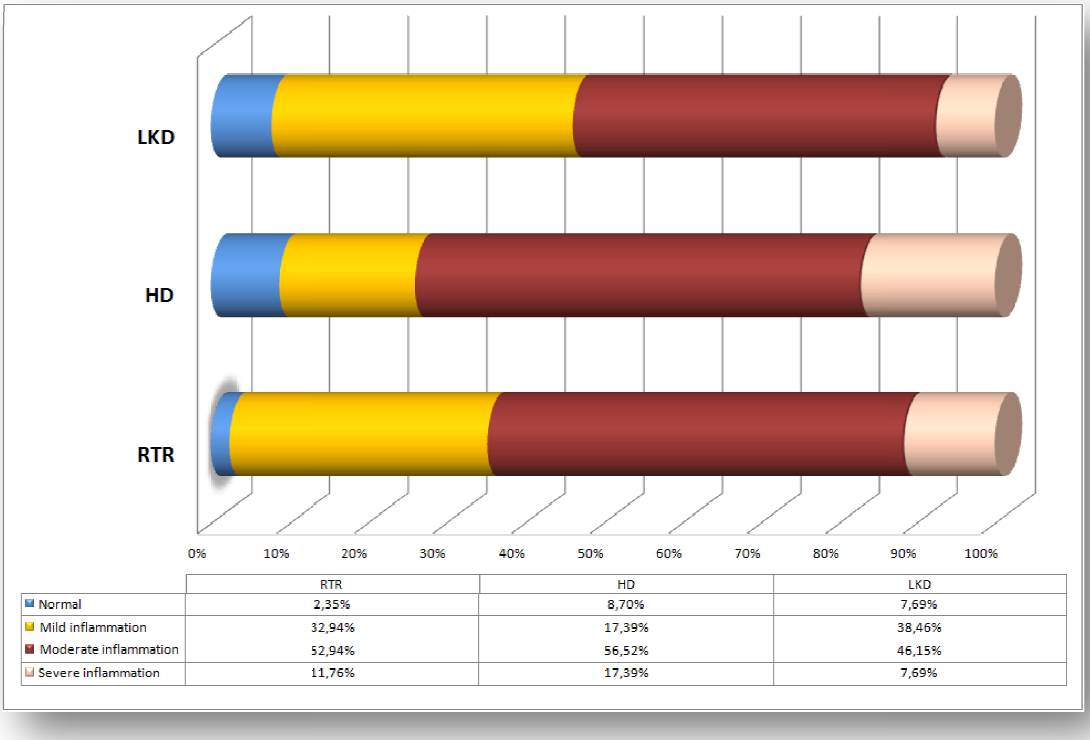


Figure 11. Gingival enlargement.



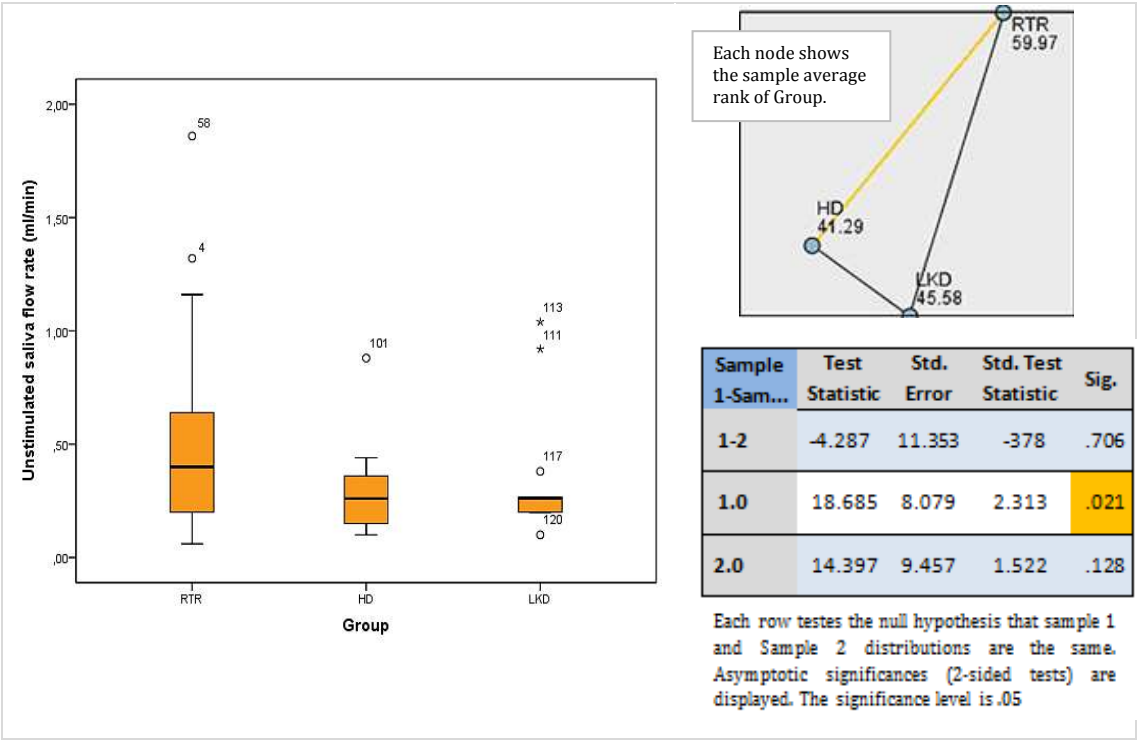
	RTR	HD	LKD
no	85,06%	94,44%	100,00%
any	14,94%	5,56%	0,00%

Figure 12. Distribution gingival enlargement by group.



	RTR	HD	LKD
Normal	2,35%	8,70%	7,69%
Mild inflammation	32,94%	17,39%	38,46%
Moderate inflammation	52,94%	56,52%	46,15%
Severe inflammation	11,76%	17,39%	7,69%

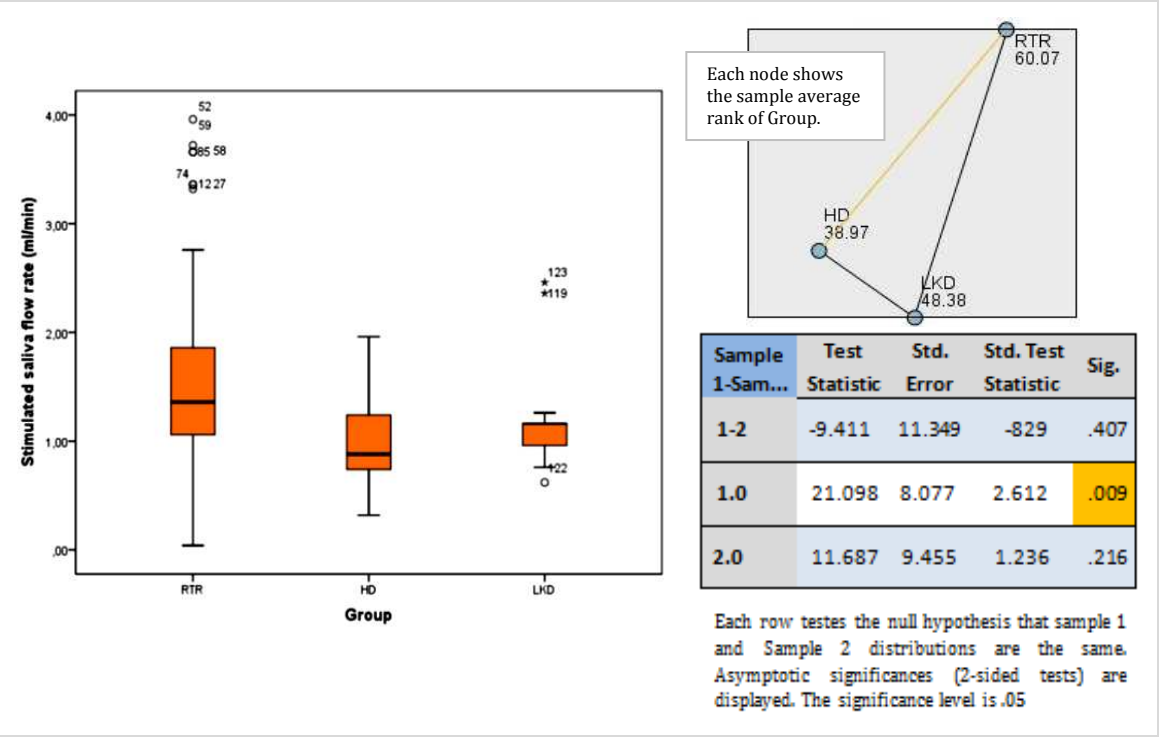
Figure 13. Distribution of gingival index scores by group.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
1-2	-4.287	11.353	-378	.706
1.0	18.685	8.079	2.313	.021
2.0	14.397	9.457	1.522	.128

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

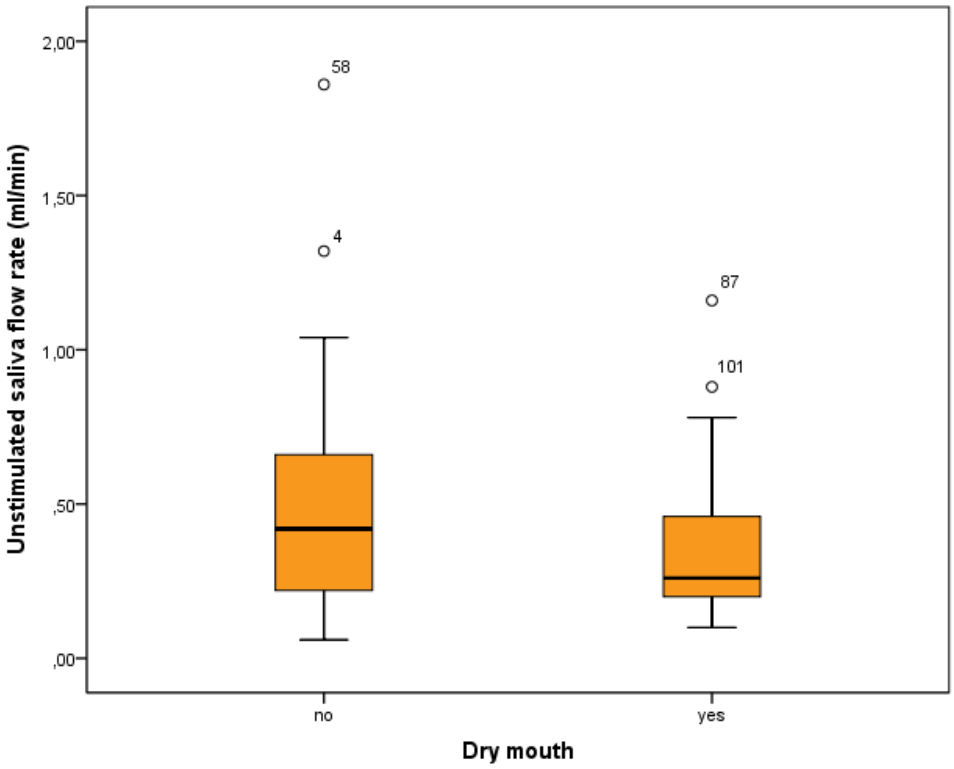
Figure 14. The distribution of unstimulated saliva flow rate by groups. Pairwise comparison of RTRs, HD patients and LKDs for unstimulated saliva flow rate.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
1-2	-9.411	11.349	-829	.407
1.0	21.098	8.077	2.612	.009
2.0	11.687	9.455	1.236	.216

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

Figure 15. The distribution of stimulated saliva flow rate by groups. Pairwise comparison of RTRs, HD patients and LKDs for stimulated saliva flow rate.



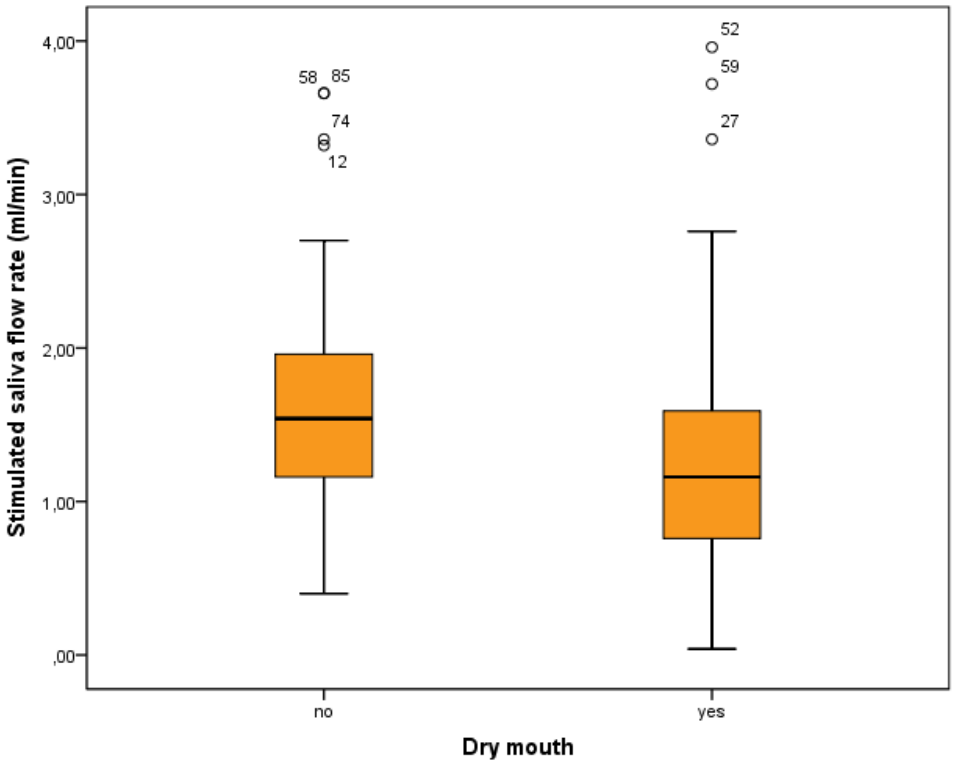


Figure 16. Distribution of unstimulated and stimulated saliva flow rate by patients with or without the feeling of a dry mouth.

The oral health evaluation data in RTRs receiving CsA, Tac and ERL, and LKD control group is given in Table 4. No significant differences were observed in oral hygiene habits and oral symptoms among the three groups (Table 4). No significant differences were found regarding visible plaque index and DMFT index among the studied groups (Table 4).

Statistically significant differences were observed in bleeding on probing among the three RTRs groups (Table 4). Through multiple comparisons tests are observed differences in bleeding on probing. These differences reveal that ERL present median values significantly lower when compared with RTRs receiving CsA. These results are illustrated in the graphs of Figure 17.

Concerning clinical attachment level, positive cases for gingival enlargement were excluded, and no differences were found among the three groups (Table 4). In addition, both unstimulated and stimulated saliva, flow rate and pH, did not differ among all studied groups (Table 4).

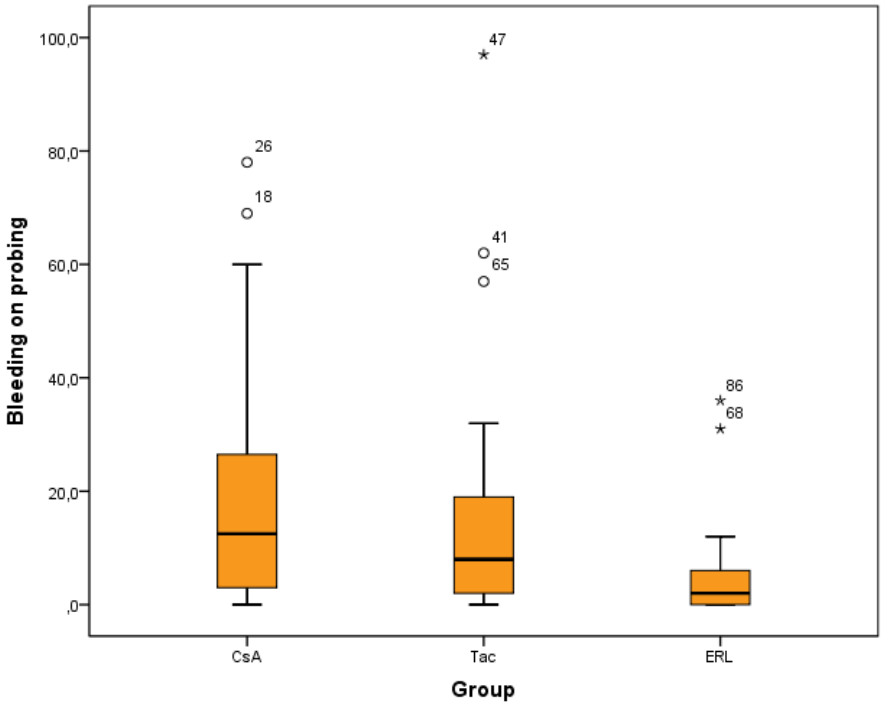
Concerning GI and GE, no statistical analysis could be performed due to the reduced number of cases distributed by group.

Table 4. Oral hygiene habits, oral symptoms, teeth evaluation, periodontal evaluation and salivary evaluation of RTRs receiving CsA, Tac and ERL.

	CsA (n=29)	**Tac** (n=36)	**ERL** (n=23)	**p**
Oral hygiene habits				
Daily tooth brushing <2 times per day	8 (33.3)	15 (46.9)	5 (26.3)	0.302*
Change toothbrush <4 times per year	12 (41.4)	10 (27.8)	11 (50.0)	0.405*
Oral symptoms				
Dry mouth	14(58.3)	20(62.5)	10(55.6)	0.809*
Bad breath	13(54.2)	18(56.3)	14(73.7)	0.366*
Teeth evaluation				
Visible Plaque index	92(86±21)	100(92±12)	87(82±23)	0.099†
Decayed	1 (2±4)	2 (3±4)	1 (3±3)	0.321†
Missing	0 (1±2)	0 (1±2)	1 (5±5)	0.120†
Filled	7 (8±7)	4 (6±6)	6 (7±5)	0.386†
DMFT index	13 (12±7)	10 (11±7)	12(11±6)	0.812†
Periodontal evaluation				
Bleeding on probing	12.5 (19.7±22.2)	8 (15±20.7)	2 (5.5±9.6)	**0.004†**
Clinical attachment level	3.2 (3.1±0.9)	2.6 (2.8±0.6)	2.8 (2.9±0.7)	0.059†
Salivary evaluation				
Unstimulated saliva flow rate (ml/min)	0.48 (0.47±0.28)	0.40 (0.43±0.33)	0.34 (0.41±0.27)	0.550†
Unstimulated saliva pH	7.4 (7.14±0.49)	7.4 (7.11±0.46)	7.4 (7.2±0.61)	0.428†
Stimulated saliva flow rate (ml/min)	1.36 (1.53±0.86)	1.32 (1.59±0.9)	1.28 (1.51±0.9)	0.866†
Stimulated saliva pH	8.0 (7.75±0.47)	8.0 (7.72±0.4)	8.0 (7.8±0.27)	0.764†

Values are presented as n (%) or median (mean ± standard deviation).

†Testing of group differences by Kruskall-Wallis test or * chi-square test.



Sample 1-Sam.	Test Statistic	Std. Error	Std. Test Statistic	Sig.
1-2	15.588	6.684	-2.332	.020
1.0	20.117	6.999	2.784	0.04
2.0	4.529	6.228	.727	.467

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

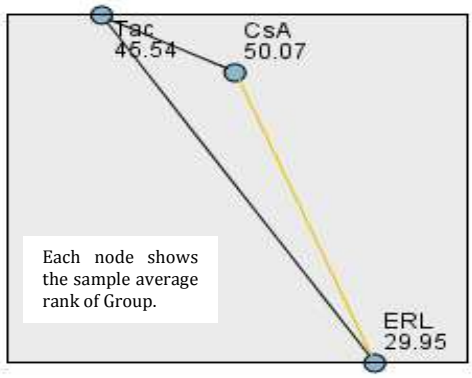


Figure 17. The distribution of bleeding on probing by group.

3 Oral fungi characterization

The prevalence of fungi isolated from whole saliva (stimulated) of RTR receiving CsA, Tac or ERL, and HD patients and LKD as group control are presented in Table 5. The prevalence of fungi isolated from whole saliva (stimulated) in RTRs receiving CsA, Tac and ERL were compared to HD patients and LKD (Table 5). No differences were found among all studied groups (Table 5).

Table 5. Prevalence of fungi isolated from whole saliva (stimulated) among RTRs receiving CsA, Tac and ERL, HD and LKDs groups.

	CsA	Tac	ERL	HD	LKD	p
Fungi prevalence (%)	10 (37)	15 (52)	8 (36)	9 (41)	5 (38)	0.780
Fungi quantification log CFU/ml	2.67±0.22	2.77±0.15	2.48±0.17	2.58±0.25	2.84±0.38	0.830

Values are presented as number (%) and as mean ± standard deviation. Testing of group differences by *chi-square test or #one-way ANOVA.

Identification of *Candida albicans* and *Candida krusei* was possible due to the specific colour of the colonies, green and pink respectively) – Figures 18 and 19.

Candida *albicans* was the most prevalent species identified among the studied groups (Figure 20). Besides C. *albicans*, C. *krusei* and C. *parapsilosis* were also detected in the studied groups (Figure 20). Although the incubation conditions have been specific to yeasts, molds were also found in all studied groups (Figure 20).

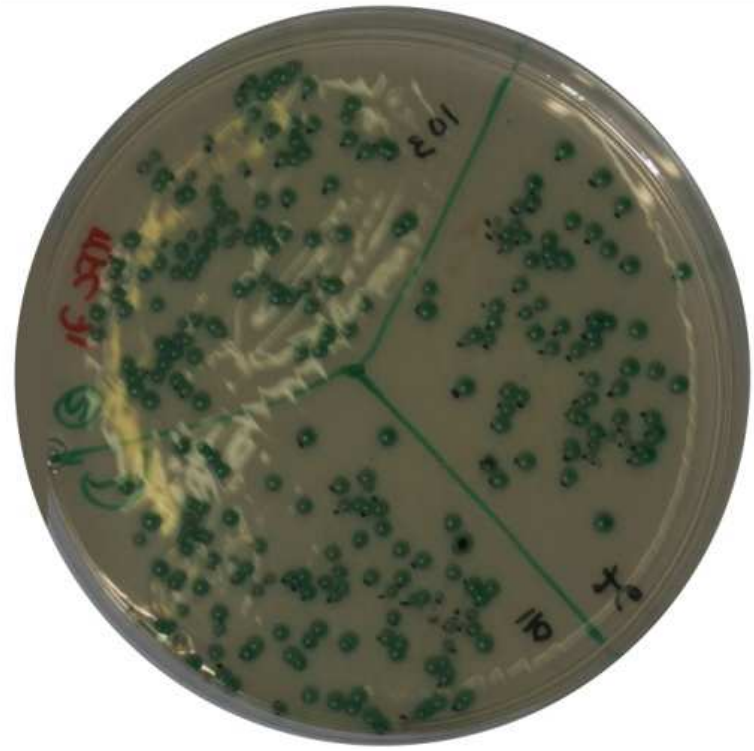


Figure 18. Green colonies of Candida *albicans.*

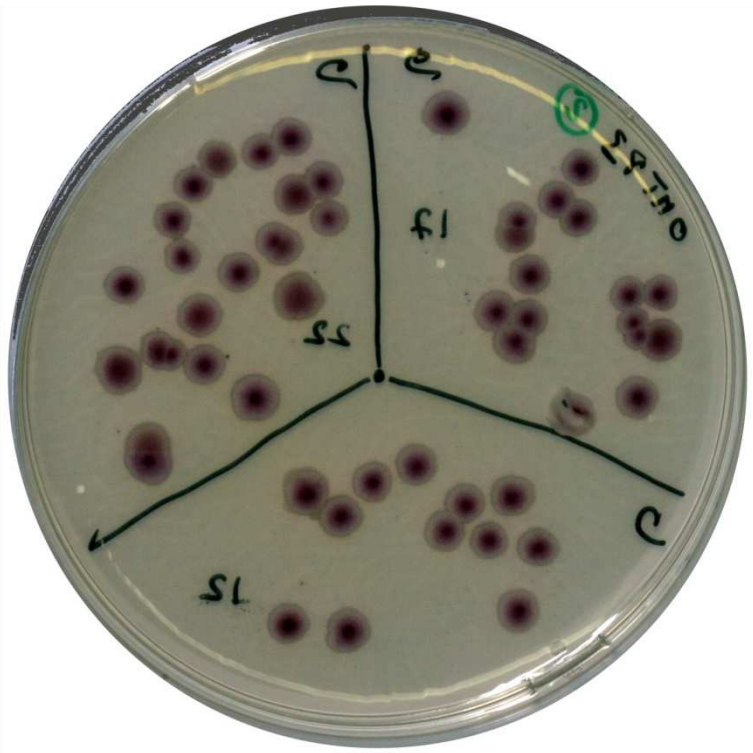


Figure 19. Pink colonies of Candida *krusei.*

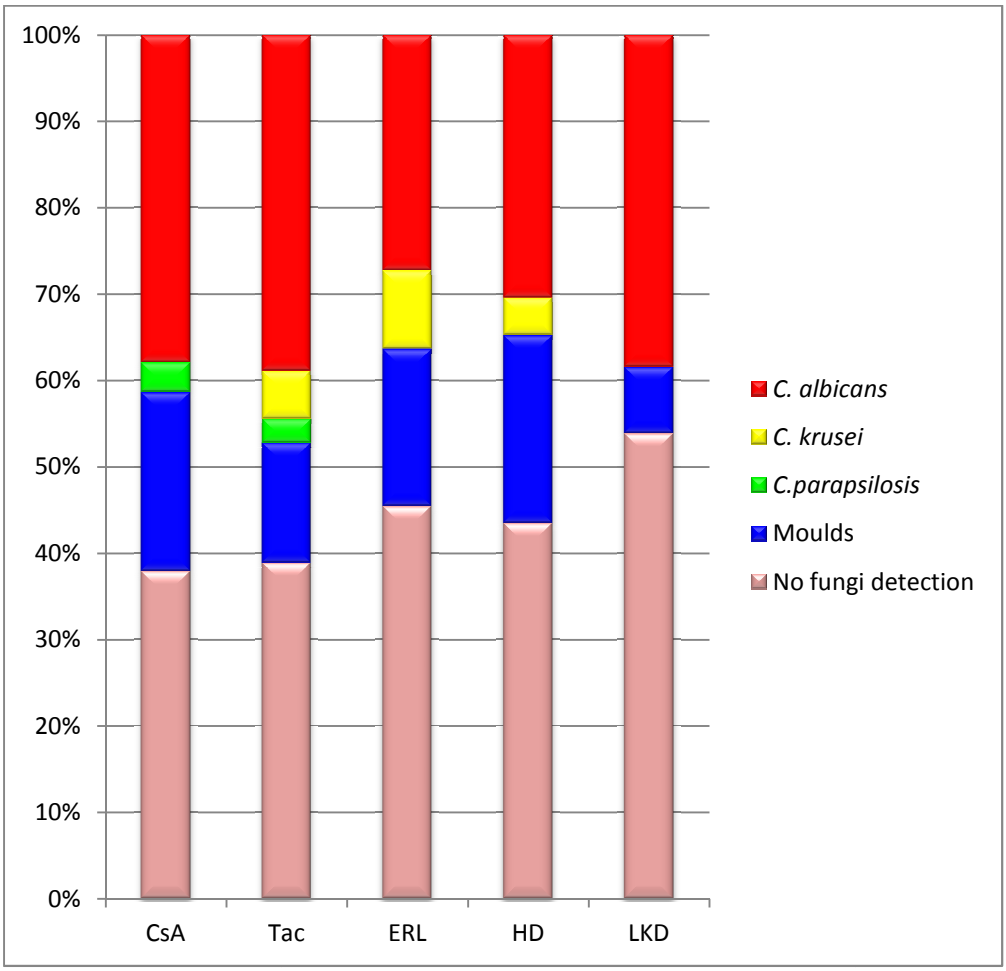


Figure 20. Fungi isolated from whole saliva of RTRs receiving CsA, Tac and ERL, HD patients and LKDs groups.

4 Saliva analysis

The composition of saliva in RTRs, HD and LKDs groups is given in Table 6.

Concerning unstimulated saliva, statistically significant differences were observed in potassium levels among the three groups (Table 6). Through multiple comparisons tests are observed differences in potassium levels. These differences revealed that HD patients presented median values significantly higher when compared with RTRs. These results are illustrated in the graphs of Figure 21. No significant differences were observed in chloride, calcium, sodium, magnesium, iron and phosphate among the three groups (Table 6).

Statistically significant differences were observed in urea, uric acid and creatinine levels among the three groups (Table 6). Through multiple comparisons tests are observed differences in urea, uric acid and creatinine levels. These differences reveal that HD patients presented median values of urea significantly higher when compared with RTRs and LKDs, these results are illustrated in the graphs of Figure 22; median values of uric acid significantly higher when compared with RTRs, these results are illustrated in the graphs of Figure 23; median values of creatinine significantly higher when compared with RTRs and LKDs, these results are illustrated in the graphs of Figure 24.

Statistically significant differences were observed in LDL and triglycerides among the three groups (Table 6). Through multiple comparisons tests are observed differences in LDL and triglycerides levels. These differences reveal that HD patients present median values of LDL significantly higher when compared with RTRs, these results are illustrated in the graphs of Figure 25. And, RTRs present median values of triglycerides significantly lower when compared with LKDs, these results are illustrated in the graphs of Figure 26.

No significant differences were observed in total proteins, CRP and albumin among the three groups (Table 6). Statistically significant differences were observed in AST among the three groups (Table 6). Through multiple comparisons tests are observed differences in AST levels. These differences reveal that HD patients present median values of AST significantly higher when compared with RTRs, these results are illustrated in the graphs of Figure 27. Statistically significant differences were observed in LDH among the three groups (Table 6). Through multiple comparisons tests are observed differences in LDH levels. These differences reveal that RTRs present median values of LDH significantly lower when compared with LKDs, these results are illustrated in the graphs of Figures 28. Statistically significant differences were observed in ALP among the three groups (Table 6). Through multiple comparisons tests are observed differences in ALP levels. These differences reveal that HD patients present median values of ALP significantly higher when compared with RTRs. Additionally, RTRs present median

values of ALP significantly higher when compared with LKDs, these results are illustrated in the graphs of Figures 29.

Concerning stimulated saliva, statistically significant differences were observed in potassium levels among the three groups (Table 6). Through multiple comparisons tests are observed differences in potassium levels. These differences reveal that HD patients present median values significantly higher when compared with RTRs and LKDs. These results are illustrated in the graphs of Figure 30. No significant differences were observed in chloride, magnesium, iron, phosphate and α-amylase levels among the three groups (Table 6).

Statistically significant differences were observed in IgA and s-IgA among the three groups (Table 6). Through multiple comparisons tests are observed differences in IgA and s-IgA levels. These differences reveal that HD patients present median values of IgA and s-IgA significantly higher when compared with RTRs. These results are illustrated in the graphs of Figures 31 and 32.

Statistically significant differences were observed in IgG among the three groups (Table 6). HD patients present median values of IgG significantly higher when compared with RTRs. These results are illustrated in the graph of Figure 33.

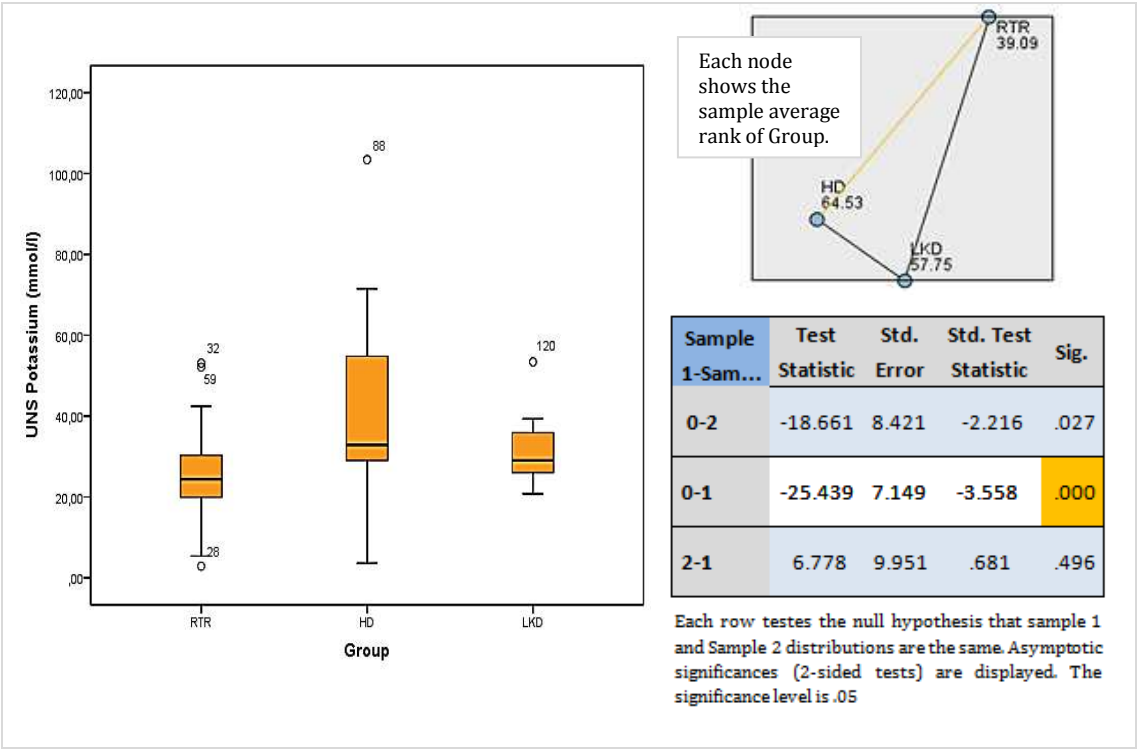
Table 6. Saliva composition in RTRs, HD and LKDs groups.

Unstimulated Saliva	RTR	HD	LKD	p
Electrolytes				
Potassium (mmol/l)	24.40 (25.17±9.4)	32.90 (40.47±22.62)	29.05 (31.83±8.83)	**0.001**
Chloride (mmol/l)	55.80 (62.96±39.26)	58.85 (59.55±20.83)	62.85 (79.53-33.56)	0.132
Calcium (mg/dl)	2.86 (4.14±3.28)	3.42 (5.69-4.89)	3.55 (4.49-3.87)	0.592
Sodium (mg/dl)	52.8 (60.4±56.1)	44.45 (46.59±43.82)	52.45 (47.69-33.69)	0.843
Magnesium (mmol/l)	0.14 (0.2±0.2)	0.15 (1.98-6.16)	0.15 (1.61±4.6)	0.395
Iron (mg/L)	0.02 (0.09±0.21)	0.04 (0.16±0.24)	0.15 (0.16±0.14)	0.159
Phosphate (mg/dl)	16.67 (16.83±4.24)	18.78 (23.18±16.32)	15.21 (20.56±19.6)	0.274
Organic Part				
Urea (mg/dl)	75.37 (84.01±33.41)	155.05 (169.4±161.39)	54.33 (108.89-142.68)	**<0.001**
Uric acid (mg/dl)	3.72 (4.45±2.69)	2.02 (2.34±2.04)	2.86 (3.09±2.17)	**0.010**
Creatinine (mg/dl)	0.1 (0.2±0.18)	0.82 (1.03±0.68)	0.1 (0.15±0.1)	**<0.001**
Lipid Profile				
Cholesterol-LDL (U/L)	0.25 (1±2.78)	0.79 (4±6.41)	0.3 (3.12±6.43)	**0.008**
Triglycerides (mg/dl)	8 (23.22±32.81)	23.19 (48±56.93)	60.01 (60.61±26.24)	**0.009**
Proteins				
Total proteins (mg/Ll)	0.7 (579.65±860.54)	2.33 (766.65±1209.29)	1030 (960.87±1063.68)	0.364
C-reactive protein (mg/l)	0.03 (28.97±68.57)	0.02 (0.08±0.21)	0.03 (0.1±0.2)	0.081
Albumin (mg/l)	47.3 (63.94±53.04)	44.7 (44.02±32.6)	29.45 (56.43±59.93)	0.771

Table 6. Saliva composition in RTRs, HD and LKDs groups.

Unstimulated Saliva	RTR	HD	LKD	p
Aspartate Aminotransferase (U/l)	32.9 (50.02±62.8)	74.9 (119.55±111.74)	28 (50.86±54.49)	**0.003**
Lactate dehydrogenase (U/l)	83 (124.23±118.55)	271.5 (258.9±178.29)	212 (327.2±293.38)	**0.011**
Alkaline phosphatase (U/l)	7.5 (25.38-77.74)	18.7 (52.66-90.72)	5.15 (11.12-14.29)	**0.005**
Stimulated Saliva				
Electrolytes				
Potassium (mmol/l)	20.70 (21.64±5.13)	27.80 (29.90±12.49)	21.80 (21.78±8.87)	**<0.001**
Chloride (mmol/l)	22.35 (25.99±12.99)	23.30 (25.83±13.63)	24.85 (35.58±34.93)	0.949
Magnesium (mmol/l)	0.06 (0.09±0.13)	0.09 (0.15±0.15)	0.08 (0.09±0.05)	0.139
Iron (mg/l)	0.01 (0.02±0.02)	0.02 (0.04±0.04)	0.01 (0.01±0.01)	0.067
Phosphate (mg/dl)	13.16 (13.92±3.58)	17.41 (16.86±5.55)	15.52 (15.39±4.23)	0.055
Proteins				
α-amylase (U/l)	129.3 (226.45±276.25)	92.30 (206.42±241.64)	107.60 (115.66±65.18)	0.288
Immunoglobins				
Immunoglobulin A (U/l)	0.06 (0.08±0.07)	0.08 (0.12±0.09)	0.08 (0.08±0.04)	**0.012**
Immunoglobulin G (U/l)	0.02(0.03±0.04)	0.03(0.05±0.05)	-	**0.049**

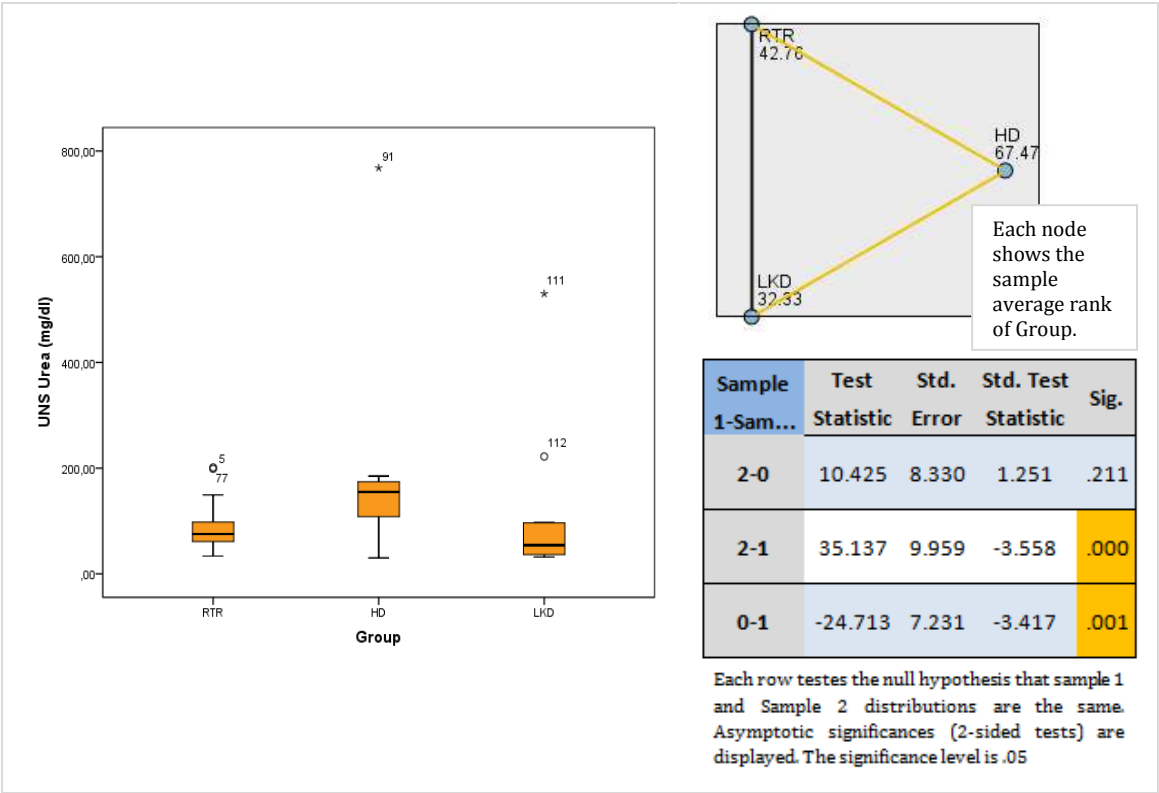
Values are presented as median (mean±standar deviation). Testing of group differences by Kruskall-Wallis test.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
0-2	-18.661	8.421	-2.216	.027
0-1	-25.439	7.149	-3.558	.000
2-1	6.778	9.951	.681	.496

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

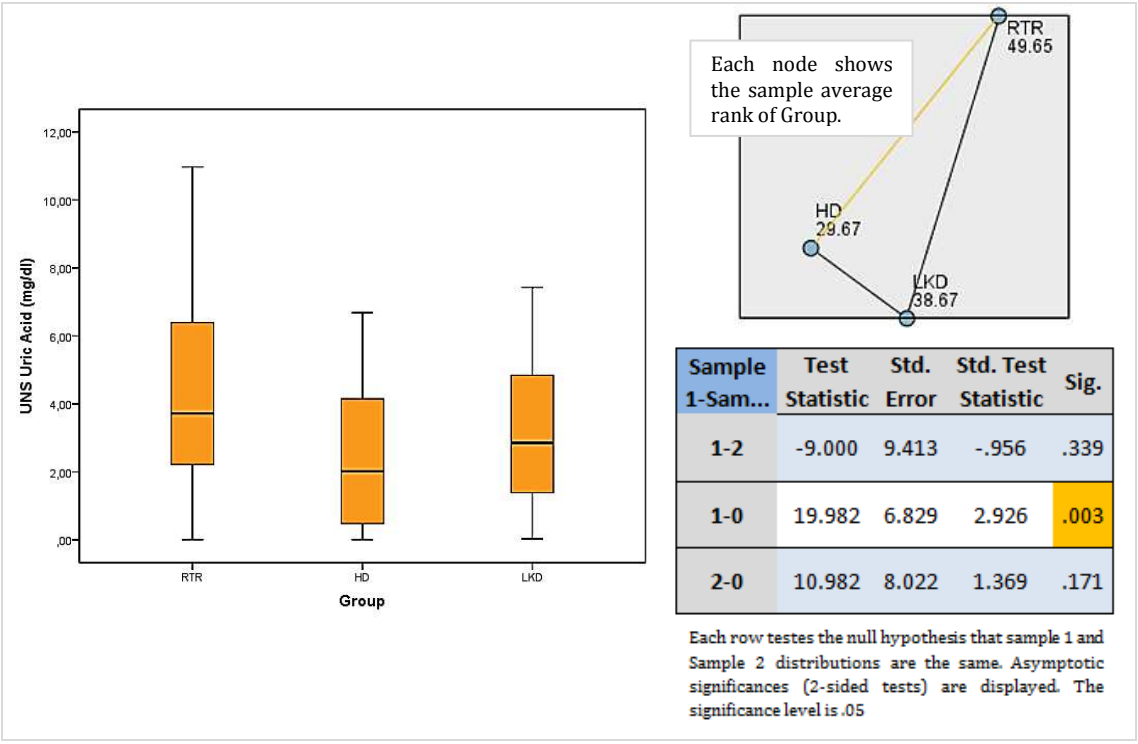
Figure 21. The distribution of potassium in unstimulated saliva by group. Pairwise comparisons of RTRs, HD and LKDs groups for potassium in unstimulated saliva.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
2-0	10.425	8.330	1.251	.211
2-1	35.137	9.959	-3.558	.000
0-1	-24.713	7.231	-3.417	.001

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

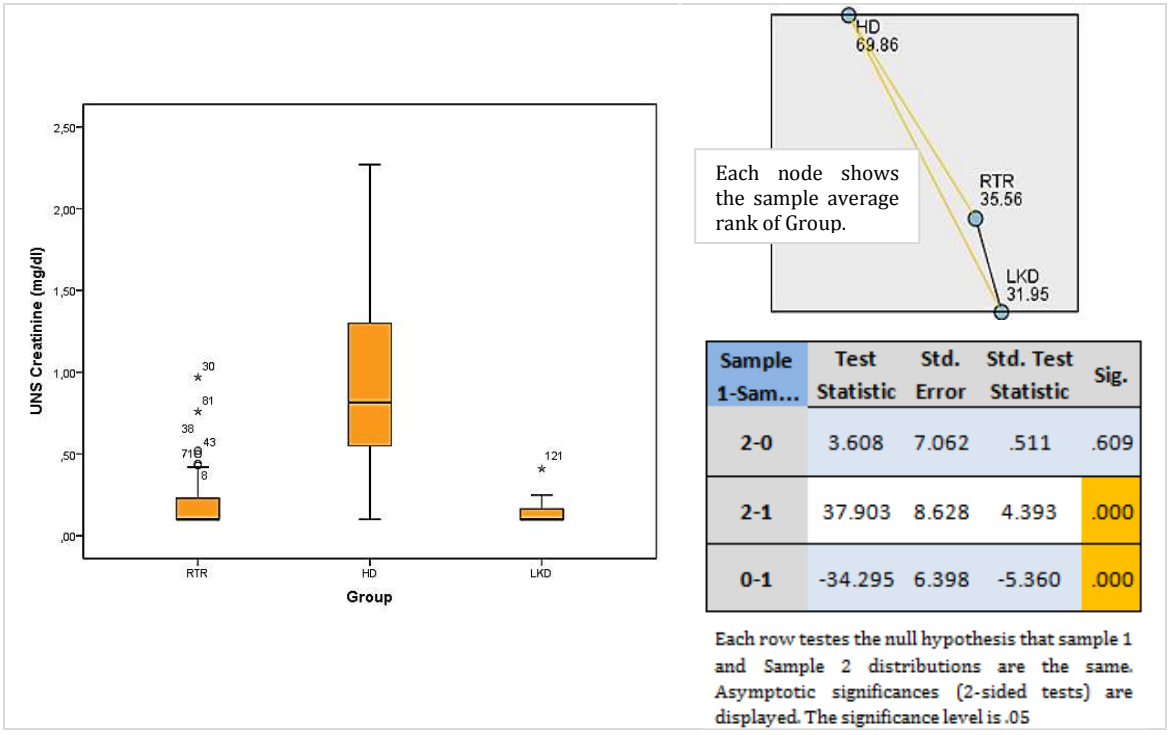
Figure 22. The distribution of urea in unstimulated saliva by group. Pairwise comparisons of RTRs, HD and LKDs groups for urea in unstimulated saliva.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
1-2	-9.000	9.413	-.956	.339
1-0	19.982	6.829	2.926	.003
2-0	10.982	8.022	1.369	.171

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

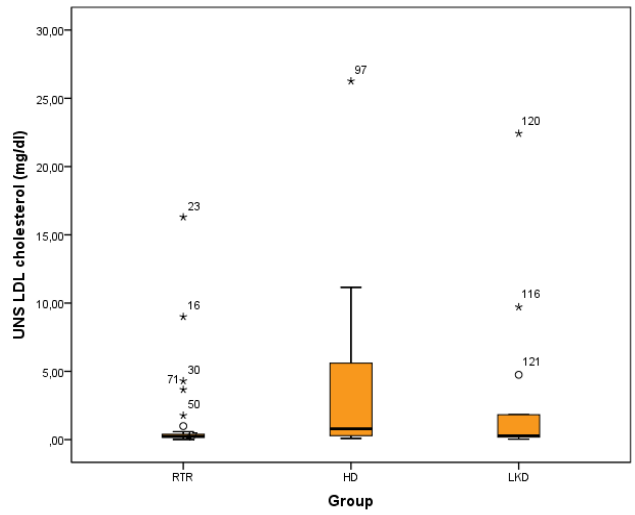
Figure 23. The distribution of uric acid in unstimulated saliva by group. Pairwise comparisons of RTRs, HD and LKDs groups for uric acid in unstimulated saliva.

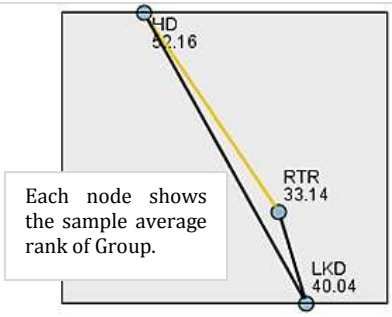


Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
2-0	3.608	7.062	.511	.609
2-1	37.903	8.628	4.393	.000
0-1	-34.295	6.398	-5.360	.000

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

Figure 24. The distribution of creatinine in unstimulated saliva by group. Pairwise comparisons of RTRs, HD and LKDs groups for creatinine in unstimulated saliva.

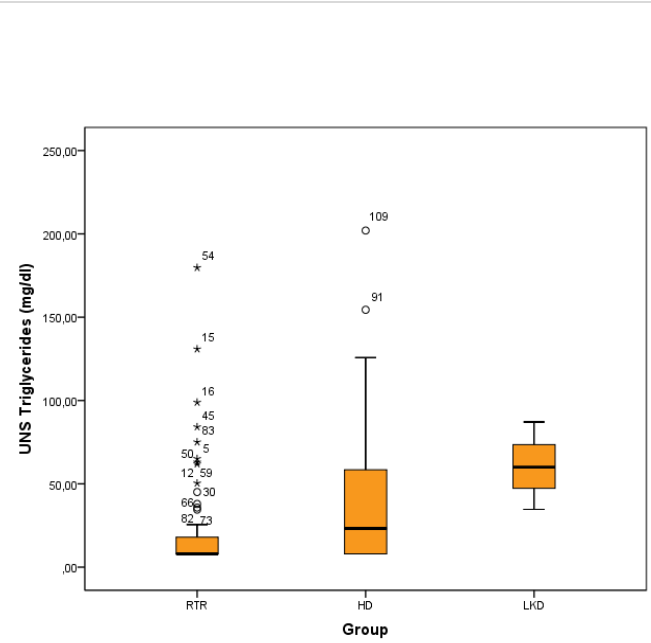


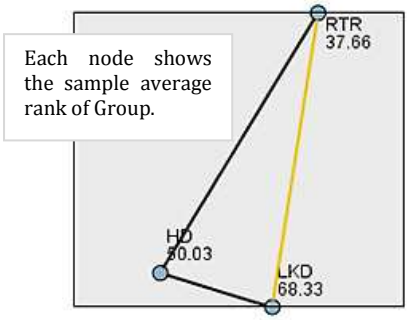


Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
0-2	-6.894	7.043	-.979	.328
0-1	-19.013	6.119	-3.107	.002
2-1	12.119	8.050	1.505	.132

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

Figure 25. The distribution of LDL in unstimulated saliva by group. Pairwise comparisons of RTRs, HD and LKDs groups for LDL in unstimulated saliva.

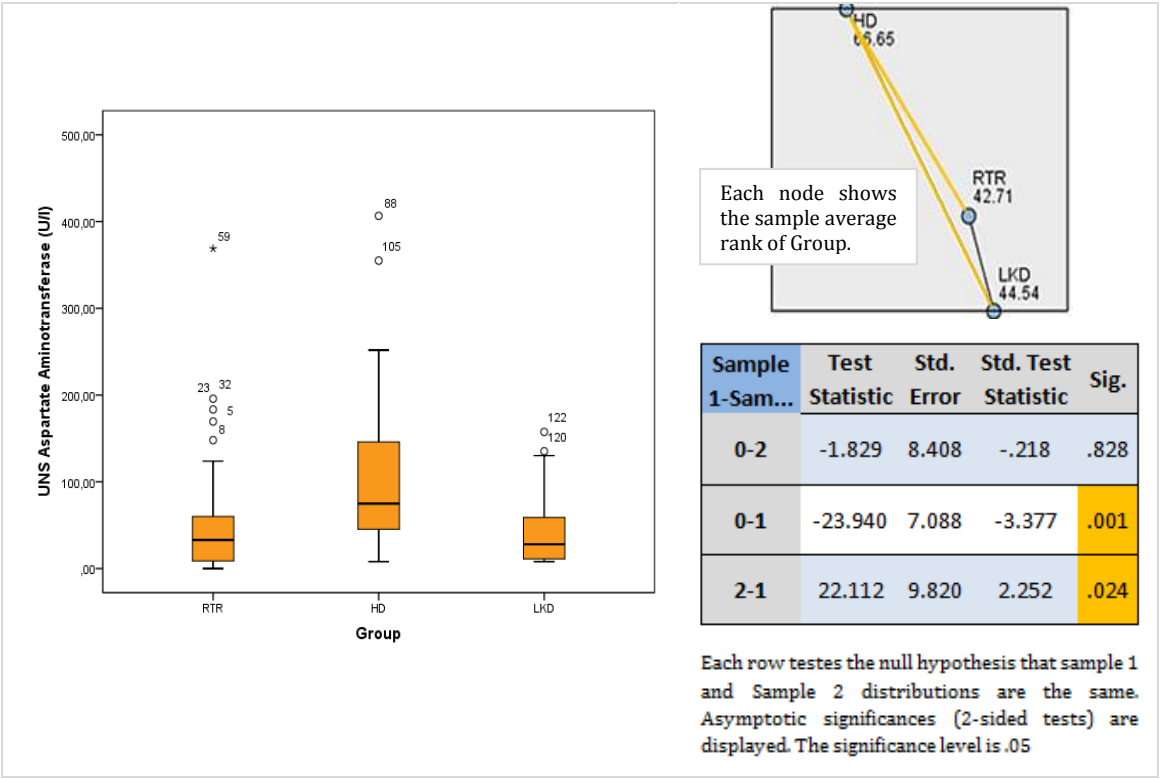




Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
0-1	-12.364	5.797	-2.133	.033
0-2	-30.669	12.781	-2.400	.016
1-2	18.306	13.477	-1.358	.174

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

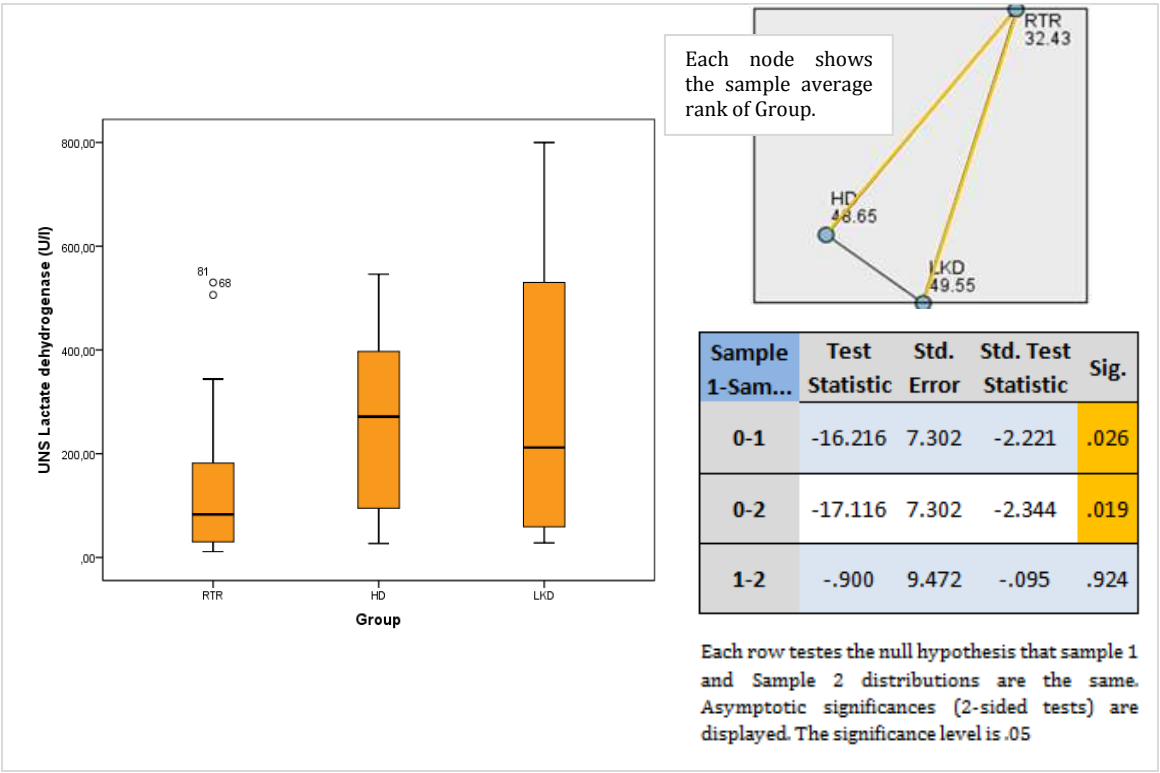
Figure 26. The distribution of triglycerides in unstimulated saliva by group. Pairwise comparisons of RTRs, HD and LKDs groups for triglycerides in unstimulated saliva.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
0-2	-1.829	8.408	-.218	.828
0-1	-23.940	7.088	-3.377	.001
2-1	22.112	9.820	2.252	.024

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

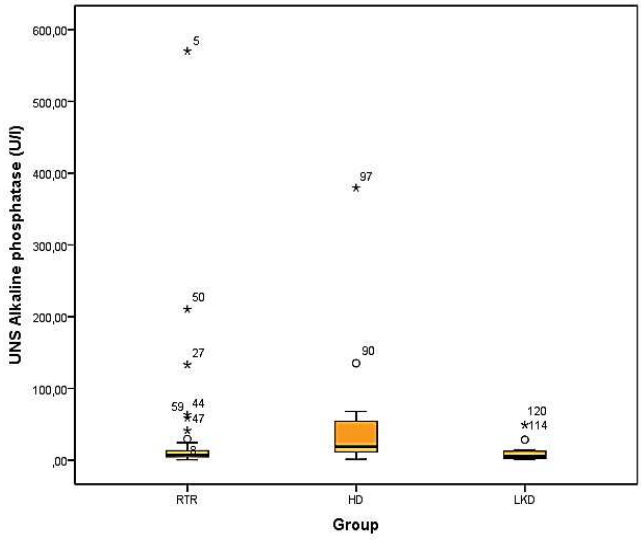
Figure 27. The distribution of AST in unstimulated saliva by group. Pairwise comparisons of RTRs, HD and LKDs groups for AST in unstimulated saliva.

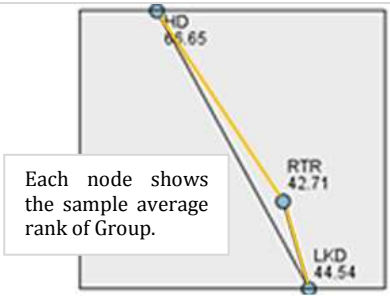


Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
0-1	-16.216	7.302	-2.221	.026
0-2	-17.116	7.302	-2.344	.019
1-2	-.900	9.472	-.095	.924

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

Figure 28. The distribution of LDH in unstimulated saliva by group. Pairwise comparisons of RTRs, HD and LKDs groups for LDH in unstimulated saliva.



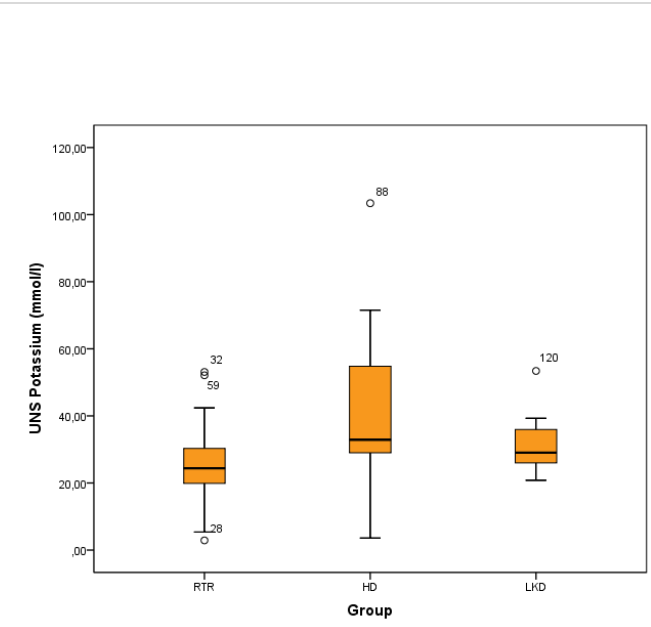


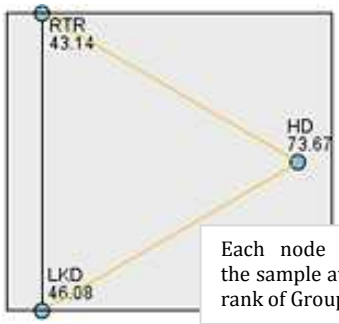
Each node shows the sample average rank of Group.

Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
2-0	7.374	8.421	.876	.381
2-1	24.861	9.951	2.498	.012
0-1	-17.487	7.149	-2.446	.014

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

Figure 29. The distribution of ALP in unstimulated saliva by group. Pairwise comparisons of RTRs, HD and LKDs groups for ALP in unstimulated saliva.





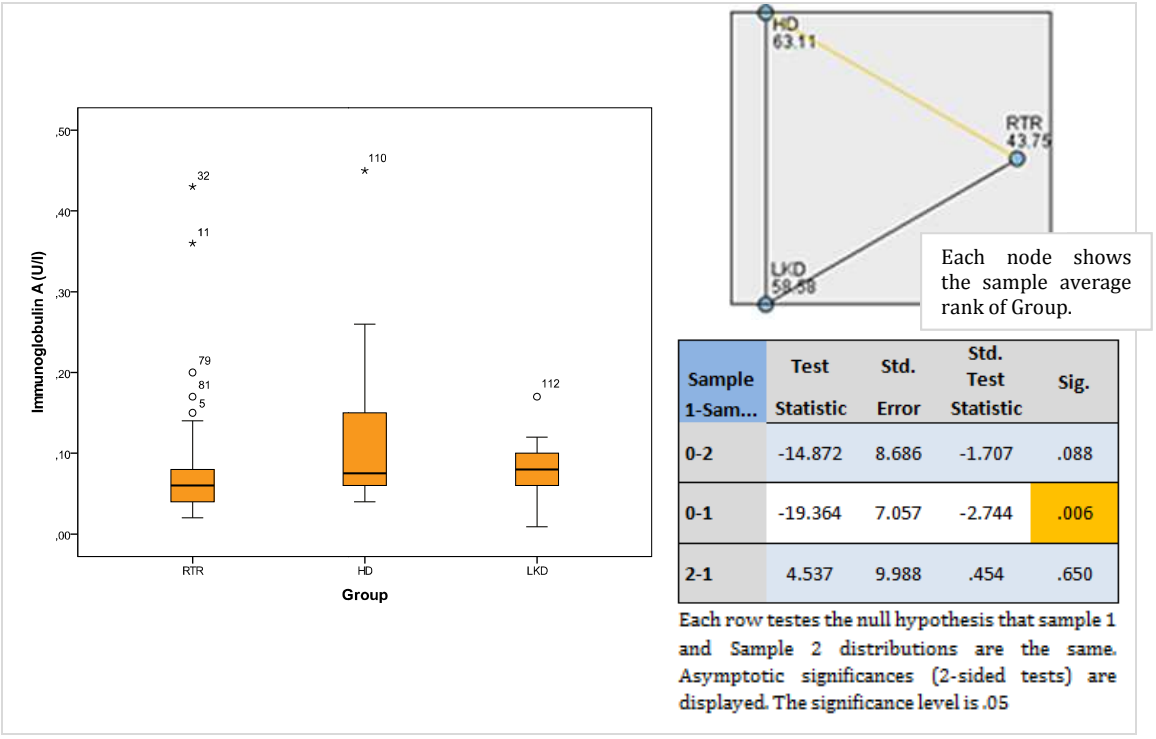
Each node shows the sample average rank of Group.

Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
0-2	-2.938	8.726	-.337	.736
0-1	-30.528	7.209	-4.235	.000
2-1	27.590	10.135	2.722	.005

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

Figure 30. The distribution potassium in stimulated saliva by group. Pairwise comparisons of RTRs, HD and LKDs groups for potassium in stimulated saliva.

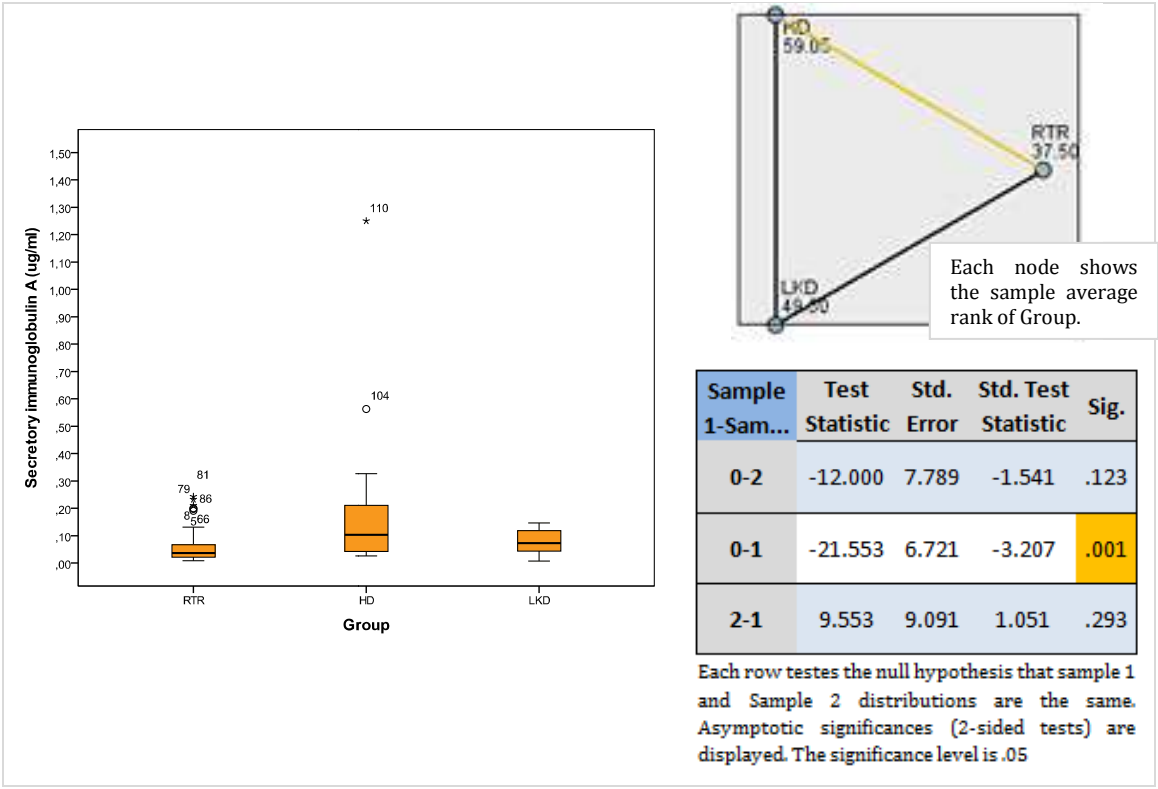
Results



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
0-2	-14.872	8.686	-1.707	.088
0-1	-19.364	7.057	-2.744	.006
2-1	4.537	9.988	.454	.650

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

Figure 31. The distribution of IgA in stimulated saliva by group. Pairwise comparisons of RTRs, HD and LKDs groups for IgA in stimulated saliva.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
0-2	-12.000	7.789	-1.541	.123
0-1	-21.553	6.721	-3.207	.001
2-1	9.553	9.091	1.051	.293

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

Figure 32. The distribution of s-IgA in stimulated saliva by group. Pairwise comparisons of RTRs, HD and LKDs groups for s-IgA in stimulated saliva.

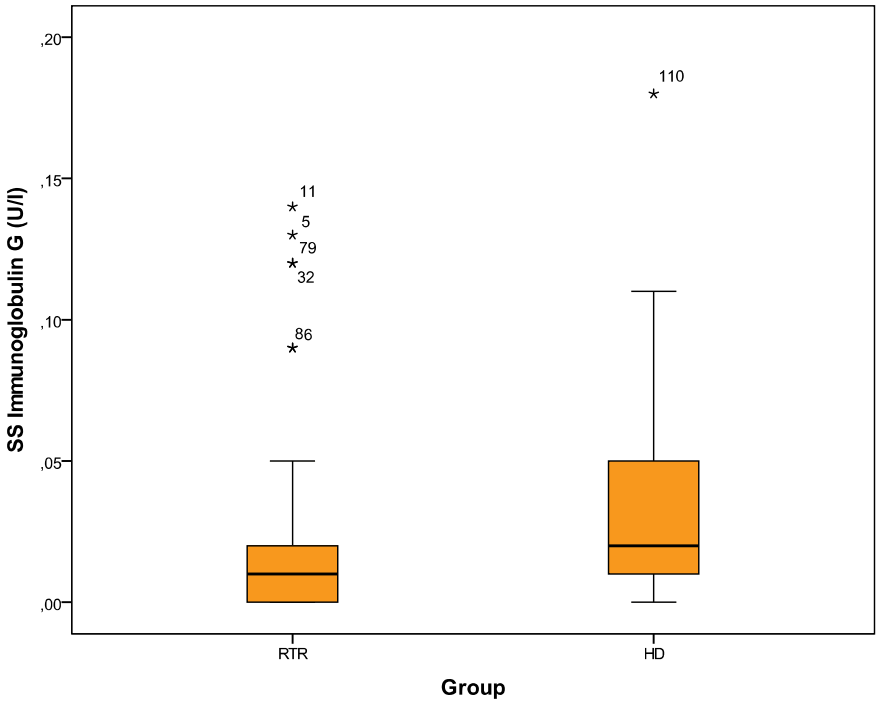


Figure 33. The distribution of immunoglobulin G in stimulated saliva by group.

The composition of saliva in RTRs receiving CsA, Tac and ERL is given in Table 7.

Concerning unstimulated saliva, statistically significant differences were observed in potassium levels among RTRs receiving CsA, Tac and ERL (Table 7). Through multiple comparisons tests are observed differences in potassium levels. These differences reveal that RTRs receiving Tac present median values significantly higher when compared with RTRs receiving CsA. These results are illustrated in the graphs of Figure 34. No significant differences were observed in chloride, calcium, sodium, magnesium and iron among the studied groups (Table 7). Statistically significant differences were observed in phosphate levels among RTRs receiving CsA, Tac and ERL (Table 7). Through multiple comparisons tests are observed differences in phosphate levels. These differences reveal that RTRs receiving CsA present median values significantly lower when compared with RTRs receiving Tac and ERL. These results are illustrated in the graphs of Figure 35.

No significant differences were observed in urea, uric acid, creatinine, LDL and triglycerides among the studied groups (Table 7).

Regarding proteins levels in unstimulated saliva, no significant differences were observed in total proteins, albumin, LDH and ALP among the studied groups (Table 7). Statistically significant differences were observed in CRP levels among RTRs receiving CsA, Tac and ERL (Table 7). Through multiple comparisons tests are observed differences in CRP levels. These differences reveal that RTRs receiving Tac present median values significantly higher when compared with RTRs receiving CsA and ERL. These results are illustrated in the graphs of Figure 36. Statistically significant differences were observed in AST levels among RTRs receiving CsA, Tac and ERL (Table 7). Through multiple comparisons tests are observed differences in AST levels. These differences reveal that RTRs receiving Tac present median values significantly lower when compared with RTRs receiving CsA and ERL. These results are illustrated in the graphs of Figure 37.

Concerning stimulated saliva, no significant differences were observed in potassium, chloride, magnesium, iron and phosphate among the studied groups (Table 7).No significant differences were observed in α-amylase, IgA, s-IgA and IgG among the studied groups (Table 7).

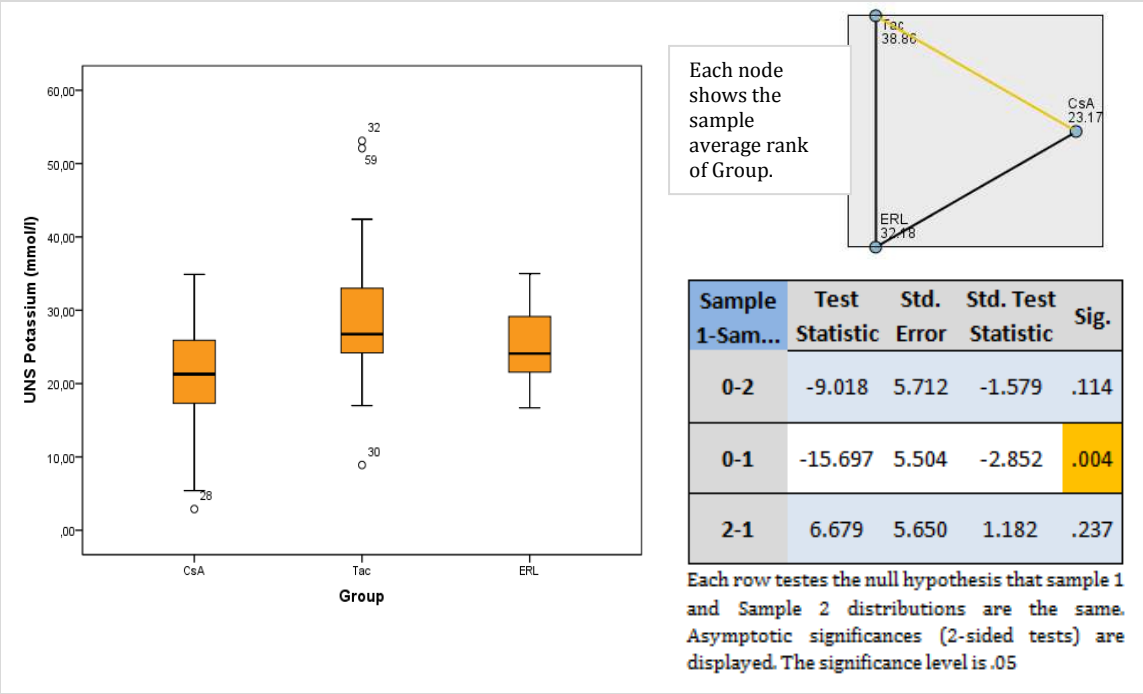
Table 7. Saliva composition of RTRs receiving CsA, Tac or ERL.

Unstimulated Saliva	CsA	Tac	ERL	p
Electrolytes				
Potassium (mmol/l)	21.30 (20.57±8.65)	26.75 (29.45±10.86)	24.10 (25.31±5.62)	**0.017**
Chloride (mmol/l)	36.50 (62.68±49.4)	61.3 (69.31±36.77)	55.8 (56.24±28.78)	0.333
Calcium (mg/dl)	2.66 (4.67±4.44)	2.72 (3.98±2.93)	3.09 (3.71±1.84)	0.654
Sodium (mg/dl)	90.9 (87.5±75.77)	34.40 (44.95±48.23)	55.4 (53.73±32.70)	0.813
Magnesium (mmol/l)	0.10 (0.2±0.28)	0.18 (0.22±0.17)	0.15 (0.18±0.11)	0.236
Iron (mg/L)	0.02 (0.16±0.32)	0.02 (0.09±0.15)	0.01 (0.02-0.03)	0.935
Phosphate (mg/dl)	13.84 (14.15±2.93)	18.90 (18.42±3.80)	19.43 (18±4.63)	**0.005**
Organic Part				
Urea (mg/dl)	68.99 (77.27±33.71)	85.53 (86.07±29.49)	87.47 (88.48±37.58)	0.238
Uric acid (mg/dl)	3.23 (3.74±2.51)	3.97 (5.01±2.91)	4.89 (4.48±2.59)	0.364
Creatinine (mg/dl)	0.10 (0.14±0.09)	0.10 (0.23±0.22)	0.10 (0.22±0.18)	0.229
Lipid Profile				
Cholesterol-LDL (U/L)	0.22 (1.68±4.33)	0.16 (0.55±1.03)	0.32 (0.66±1.06)	0.206
Triglycerides (mg/dl)	8 (25.11±35.68)	8 (26.83-40.66)	8 (17.46-17.70)	0.986
Proteins				
Total proteins (mg/Ll)	0.68 (0.33-3116.00)	0.79 (0.23-3274.00)	0.59 (0.02-1474.00)	0.196
C-reactive protein (mg/l)	0.03 (0.03±0.01)	71.50 (94.07±97.25)	0.03 (0.03±0.02)	**<0.001**

Table 7. Saliva composition of RTRs receiving CsA, Tac or ERL.

Stimulated Saliva	CsA	Tac	ERL	p
Proteins				
Albumin (mg/l)	59.58 (74.67±60.64)	65.85 (64.13±44.55)	22.86 (44.42±44.54)	0.377
Aspartate Aminotransferase (U/l)	59.90 (75.34±53.90)	0.05 (33.56-83)	44 (41.50-24.14)	**<0.001**
Lactate dehydrogenase (U/l)	172.00 (149.56±99.58)	64.15 (89±79.97)	65 (136.26±155.99)	0.177
Alkaline phosphatase (U/l)	8.50 (45.14±130.45)	6.90 (22.16±45.51)	9.35 (10.16±5.39)	0.554
Electrolytes				
Potassium (mmol/l)	20.10 (20.88±5.04)	20.70 (22.16±5.65)	21.35 (22.00±4.82)	0.527
Chloride (mmol/l)	22.30 (24.25±13.88)	22.75 (27.53±11.72)	22.25 (26.40±13.70)	0.418
Calcium (mg/dl)	3.53 (3.48±0.65)	3.13 (3.45±1.33)	2.66 (2.63±0.69)	
Magnesium (mmol/l)	0.05 (0.12±0.19)	0.06 (0.09±0.08)	0.07 (0.08±0.04)	0.417
Iron (mg/l)	0.01 (0.02±0.03)	0.01 (0.02±0.02)	0.02 (0.02±0.01)	0.993
Phosphate (mg/dl)	12.06 (13.21±3.83)	14.11 (14.68±3)	12.86 (13.94±3.85)	0.658
Proteins				
α-amylase (U/l)	148.75 (214.80±202.72)	173.80 (264.90±295.11)	98.30 (195.43±332.15)	0.158
Immunoglobulins				
Immunoglobulin A (U/l)	0.06 (0.07±0.07)	0.06 (0.07±0.09)	0.07 (0.08±0.05)	0.275
Immunoglobulin G (U/l)	0.02 (0.04±0.04)	0.01 (0.03±0.03)	0.02 (0.03±0.03)	0.444

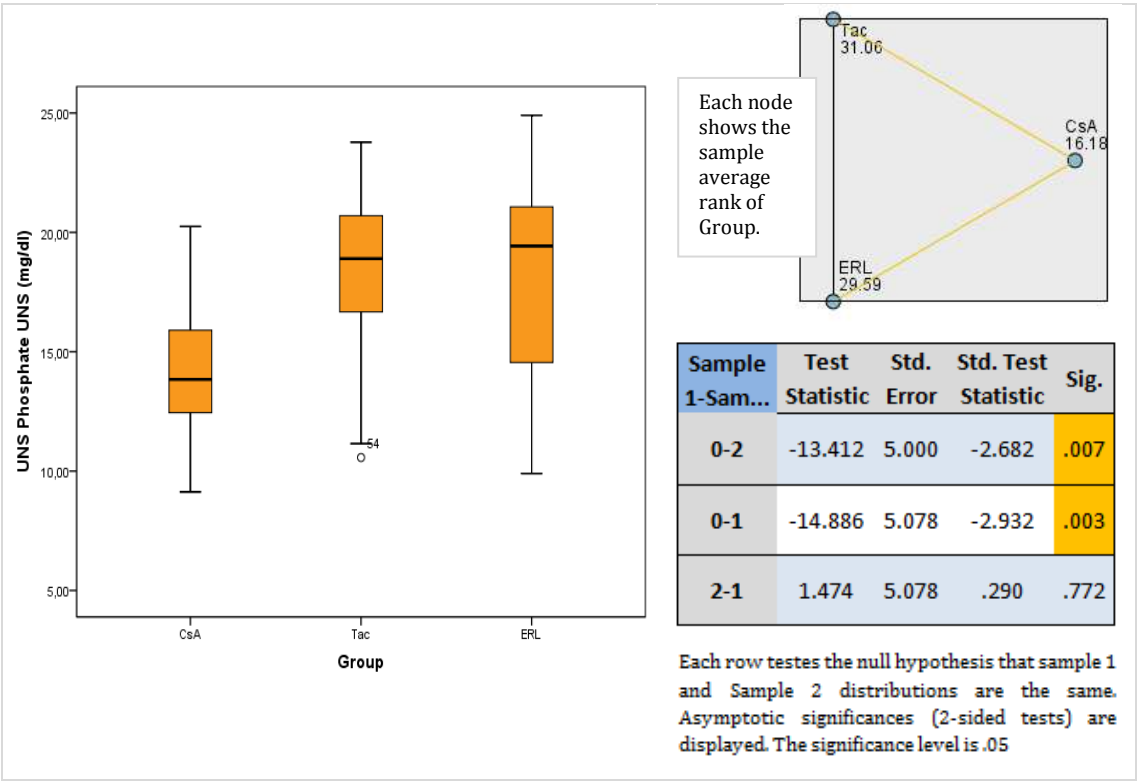
Values are presented as median (mean±standard deviation). Testing of group differences by Kruskall-Wallis test.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
0-2	-9.018	5.712	-1.579	.114
0-1	-15.697	5.504	-2.852	.004
2-1	6.679	5.650	1.182	.237

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

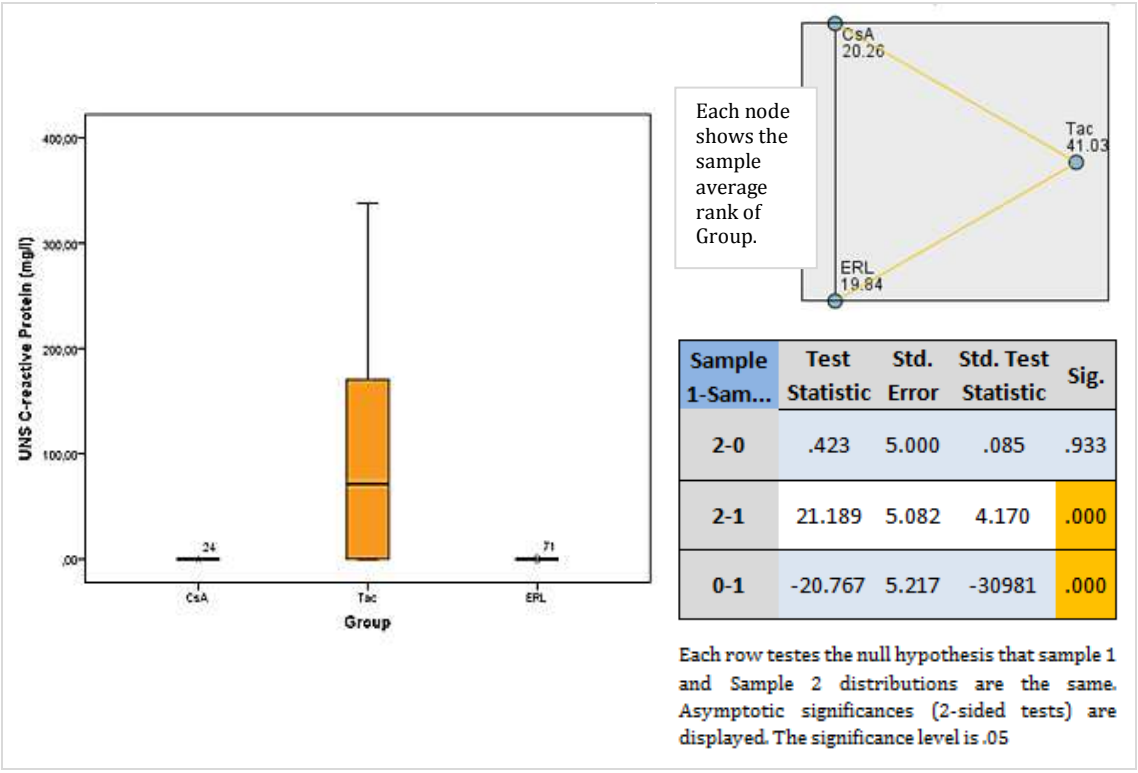
Figure 34. The distribution of potassium in unstimulated saliva by group. Pairwise comparisons of CsA, Tac and ERL groups for potassium in unstimulated saliva.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
0-2	-13.412	5.000	-2.682	.007
0-1	-14.886	5.078	-2.932	.003
2-1	1.474	5.078	.290	.772

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

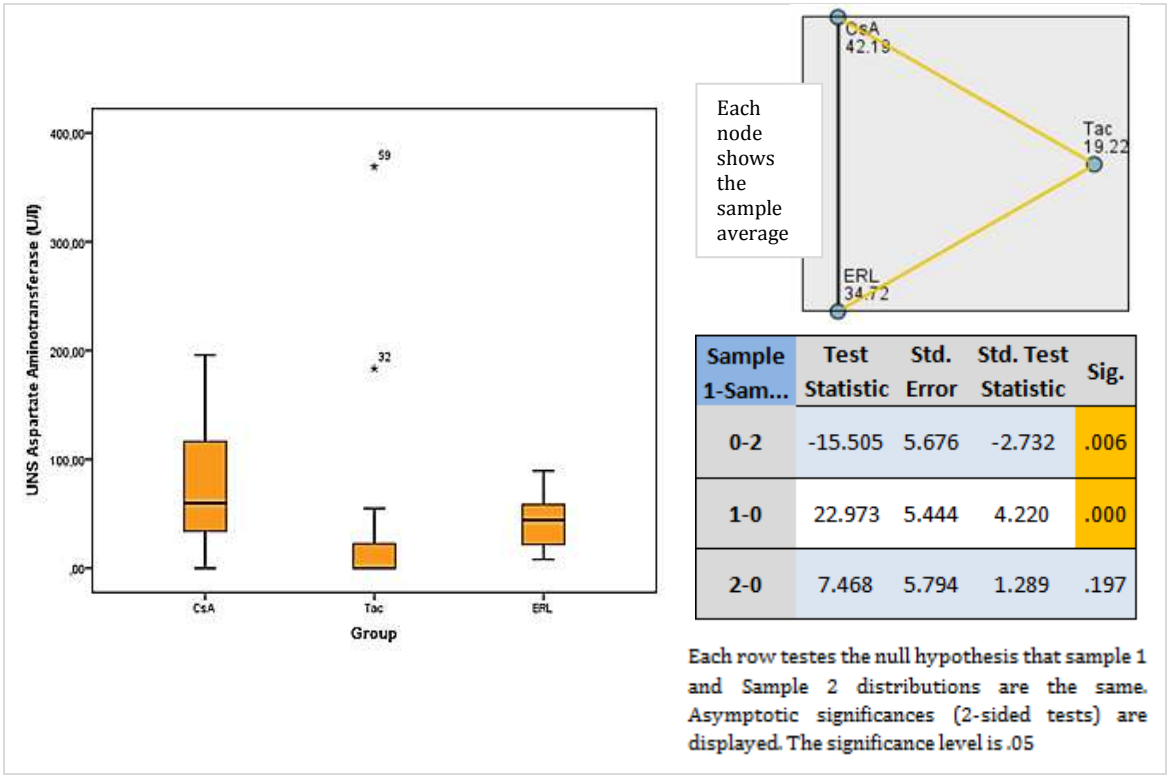
Figure 35. The distribution of phosphate in unstimulated saliva by group. Pairwise comparisons of CsA, Tac and ERL groups for phosphate in unstimulated saliva.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
2-0	.423	5.000	.085	.933
2-1	21.189	5.082	4.170	.000
0-1	-20.767	5.217	-30981	.000

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

Figure 36. The distribution of CRP in unstimulated saliva by group. Pairwise comparisons of CsA, Tac and ERL groups for CRP in unstimulated saliva.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
0-2	-15.505	5.676	-2.732	.006
1-0	22.973	5.444	4.220	.000
2-0	7.468	5.794	1.289	.197

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

Figure 37. The distribution of AST in unstimulated saliva by group. Pairwise comparisons of CsA, Tac and ERL groups for AST in unstimulated saliva.

5 Serum biochemical parameters

The serum biochemical parameters in RTRs, HD patients and LKDs group are given in Table 8. Statistically significant differences were observed in hemogram parameters such as hematocrit, leucocytes, eosinophils, lymphocytes and monocytes levels among the three groups (Table 8). Concerning general chemistry, statistically significant differences were observed in total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glucose, urea, uric acid, creatinine, calcium, sodium, potassium, chloride, inorganic phosphorus, magnesium and transferrin levels among the three groups (Table 8). Statistically significant differences were observed in the levels of total proteins, albumin, ALP and parathormone among the three groups (Table 8).

Table 8. Serum biochemical parameters assessed in RTRs, HD and LKDs groups.

	RTR	HD	LKD	P
Hemogram				
Hemoglobin (g/dl)	12.50 (13±3.6)	11.1 (11.3±1.7)	13.6 (13.6±0.8)	**<0.001**
Hematocrit (%)	38.30 (38.9±6.3)	31.9 (31.8±9.1)	40.5 (40.6±2.2)	**<0.001**
Platelets (x109/L)	206.00 (211±68)	207 (208±44)	218 (218±34)	0.862
Leucocytes (%)	8 (377±1618)	6 (6±2)	6 (7±2)	**0.023**
Neutrophils (%)	61 (61±10)	61 (54±24)	57 (57±7)	0.362
Eosinophils (%)	1.4 (1.6±1.1)	2.4 (2.4±1.6)	2.3 (3.6±3.5)	**0.016**
Basophils (%)	0.3 (0.4±0.3)	0.4 (0.4±0.2)	0.4 (0.5±0.4)	0.388
Lymphocytes (%)	25.9 (26.7±9.1)	23.0 (21.5±10.6)	30.6 (31.0±6.4)	**0.023**
Monocytes (%)	9 (9.3±2.8)	7.1 (6.7±3.9)	7.4 (7.7±1.7)	**0.004**
General Chemistry				
Total cholesterol (mg/dl)	203 (208±50)	160 (168±29)	200 (204±28)	**0.003**

Results

	RTR	HD	LKD	P
HDL cholesterol (mg/dl)	52 (56±18)	37 (42±14)	55 (57±12)	**0.003**
LDL cholesterol (mg/dl)	131 (130±33)	96 (107±38)	130 (128±23)	**0.021**
Triglycerides (mg/dl)	138 (151±72)	200 (224±135)	83 (101±47)	**0.001**
Glucose (mg/dl)	82 (81±16)	96 (113±67)	76 (72±8)	**0.002**
Urea (mg/dL)	61 (66±26)	111 (107±77)	36 (35±11)	**<0.001**
Uric acid (mg/dl)	6.8 (10.2±14.7)	8.2 (17.3±18.1)	4.1 (8.0±14.5)	**<0.001**
Creatinine (mg/dl)	1.55 (1.67±0.92)	8.75 (10.28±7.23)	0.80 (0.78±0.20)	**<0.001**
Calcium (mEq/L)	5.0 (5.0±0.4)	5.1 (6.3±2.3)	4.7 (4.6±0.7)	**0.010**
Sodium (mEq/L)	140 (140±2)	136 (136±2)	138 (136±8)	**<0.001**
Potassium (mEq/L)	4.2 (4.2±0.5)	4.7 (4.8±0.7)	4.0 (4.0±0.3)	**0.001**
Chloride (mEq/L)	106 (106±3)	98 (99±5)	103 (103±2)	**<0.001**
Inorganic Phosphorus (mg/dl)	3.0 (4.9±7.7)	4.8 (17.3±26.2)	3.6 (5.3±6.4)	**<0.001**
Iron (ug/dl)	82 (83±30)	59 (73±40)	73 (86±28)	0.263
Magnesium (mmol/l)	1.42 (1.42±0.20)	1.61 (1.74±0.45)	1.66 (1.68±0.10)	**<0.001**
Transferrin (mg/dl)	204 (212±44)	218 (225±47)	282 (267±27)	**0.006**
Ferritin (ng/ml)	127 (234±219)	431 (449±352)	80 (125±118)	0.066
Proteins				
Total proteins (mg/L)	69.7 (69.1±4.8)	71.2 (67.8±9.9)	73.1 (75.3±9.2)	**0.008**
C-reactive protein (mg/L)	2 (7±15)	4 (18±32)	2 (9±14)	0.161
Albumin (mg/L)	41.4	41.0	44.3	**0.019**

	RTR	HD	LKD	P
	(41.6±3.2)	(38.8±6.8)	(44.2±2.5)	
Aspartate aminotransferase (U/L)	20 (22±10)	20 (20±7)	18 (19±5)	0.687
Alanine aminotransferase (U/L)	17 (23±24)	17 (19±7)	13 (15±5)	0.223
Alkaline phosphatase (U/L)	72 (75±30)	115 (120±57)	51 (55±10)	**0.001**
Immunoglobulin A (mg/dl)	185 (240±190)	244 (298±180)	141 (141±123)	0.332
Immunoglobulin G (mg/dl)	926 (940±318)	987 (1052±234)	1645 (1645±686)	0.123
Immunoglobulin M(mg/dl)	95 (105±54)	73 (89±38)	155 (155±85)	0.503
Immunoglobulin E (mg/dl)	29 (161±295)	-	151 (151± -)	0.380
Complement				
Complement C3c (mg/dl)	120 (122±31)	147 (127±30)	128 (128±16)	0.710
Complement C4 (mg/dl)	23 (27±12)	34 (35±11)	22 (22±1)	0.132
Endocrinology				
Vitamin D (pg/ml)	18 (198±1048)	9 (12±8)	-	0.054
Parathormone(pg/)	100.9 (125.4±98.3)	401.8 (455.3±333.6)	50.4 (55.4±10.4)	**<0.001**

Values are presented as median (mean±standard deviation). Testing of group differences by Kruskall-Wallis test.

The serum biochemical parameters in RTRs receiving CsA, Tac and ERL group are given in Table 9. Statistically significant differences were observed in hemogram parameters such as leucocytes and monocytes levels among the three groups (Table 9). Concerning proteins, statistically significant differences were observed in the levels of total proteins and CRP (Table 9).

Table 9. Serum biochemical parameters assessed in RTRs receiving CsA, Tac or ERL.

	CsA	Tac	ERL	P
Hemogram				
Hemoglobin (g/dl)	12.8 (13.8±5.8)	12.1 (12.5±1.6)	12.6 (12.9±1.8)	0.435
Hematocrit (%)	38.3 (38.1±8.3)	37.60 (38.4±4.8)	41.3 (41.1±4.8)	0.133
Platelets (x109/L)	196 (194±58)	207 (208±51)	226 (241±97)	0.162
Leucocytes (%)	7 (7±2)	8. (375±1534)	9 (899±2571)	**0.043**
Neutrophils (%)	61 (62±10)	62 (62±9)	56 (56±10)	0.076
Eosinophils (%)	1.3 (1.5±0.9)	1.4 (1.5±1.3)	1.7 (1.9±1.1)	0.108
Basophils (%)	0.3 (0.4±0.3)	0.3 (0.3±0.2)	0.3 (0.3±0.3)	0.962
Lymphocytes (%)	25.3 (26.5±9.1)	24.6 (24.6±9.1)	29.6 (30.6±8.4)	0.090
Monocytes (%)	8.9 (9.2±3.1)	8.1 (8.6±2.6)	10.2 (10.6±2.3)	**0.017**
General Chemistry				
Total cholesterol (mg/dl)	230 (225±53)	198 (201±47)	198 (195±49)	0.115
HDL cholesterol (mg/dl)	54 (54±15)	50 (57±21)	59 (58±15)	0.600
LDL cholesterol (mg/dl)	147 (143±37)	127 (124±30)	116 (118±20)	0.168
Triglycerides (mg/dl)	135 (141±48)	131 (145±76)	181 (185±93)	0.153
Glucose (mg/dl)	86 (82±18)	85 (82±15)	77 (77±14)	0.167
Urea (mg/dL)	62 (69±32)	58 (62±21)	69 (68±24)	0.395
Uric acid (mg/dl)	7.2 (14.0±21.6)	6.6 (8.3±9.9)	6.9 (8.3±6.9)	0.484
Creatinine (mg/dl)	1.38 (1.57±0.61)	1.49 (1.66±1.17)	1.6 (1.83±0.86)	0.566

	CsA	Tac	ERL	P
Calcium (mEq/L)	4.9 (5.0±0.2)	5.0 (5.0±0.6)	5.2 (5.1±0.3)	0.168
Potassium (mEq/L)	4.1 (4.2±0.3)	4.2 (4.3±0.4)	3.9 (4.1±0.8)	0.600
Sodium (mEq/L)	139 (139±2)	140 (139±2)	141 (141±3)	0.115
Chloride (mEq/L)	107 (106±3)	106 (106±3)	106 (106±3)	0.638
Inorganic Phosphorus (mg/dl)	3.1 (6.0±9.3)	2.8 (4.2±7.1)	3.0 (4.5±6.1)	0.153
Iron (ug/dl)	79 (85±31)	85 (85±28)	72 (76±33)	0.167
Magnesium (mmol/l)	1.46 (1.46±0.16)	1.29 (1.33±0.20)	1.53 (1.54±0.20)	0.395
Transferrin (mg/dl)	209 (214±44)	200 (212±49)	222 (210±38)	0.786
Ferritin (ng/ml)	192 (233±168)	103 (215±218)	118 (272±297)	0.543
Iron /transferrin x 70.9 (%)	26 (29±14)	28 (30±12)	25 (26±10)	0.762
Proteins				
Total proteins (mg/L)	66.5 (68.1±5.1)	69.5 (68.5±4.7)	71.6 (72.0±3.6)	**0.013**
C-reactive protein (mg/L)	2 (3±3)	2 (5±11)	6 (15±25)	**0.011**
Albumin (mg/L)	41.1 (41.0±2.7)	42.9 (42.3±3.9)	41.3 (41.3±2.3)	0.064
Aspartate aminotransferase (U/L)	22 (22±6)	18 (21±14)	21 (22±6)	0.195
Alanine aminotransferase (U/L)	17 (20±10)	15 (28±35)	19 (18±6)	0.791
Alkaline phosphatase (U/L)	75 (78±27)	67 (72±32)	71 (78±33)	0.601
Immunogloblins				
Immunoglobulin A (mg/dl)	174 (185±86)	184 (228±114)	242 (343±354)	0.553
Immunoglobulin G	876	931	1035	0.851

	CsA	Tac	ERL	P
(mg/dl)	(902±270)	(939±317)	(992±407)	
Immunoglobulin M (mg/dl)	113 (100±54)	94 (109±61)	104 (101±45)	0.846
Immunoglobulin E (mg/dl)	8 (8±1)	73 (263±367)	-	0.083
Complement				
C3c Complement (mg/dl)	107 (114±21)	118 (116±31)	136 (143±34)	0.121
C4 Complement (mg/dl)	22 (21±5)	24 (26±11)	40 (35±13)	0.128
Endocrinology				
Vitamin D (pg/ml)	19 (573±1843)	17 (19±9)	15 (17±7)	0.755
Parathormone(pg/ml)	91.5 (99.0±45.7)	100.5 (135.2±118.7)	122.4 (148.5±111.5)	0.266

Values are presented as median (mean±standar deviation). Testing of group differences by Kruskall-Wallis test.

The ratio saliva/serum of biochemical parameters in RTRs, HD and LKDs groups, is given in Table 10.

Statistically significant differences were observed in potassium ratio among RTRs, HD and LKDs groups (Table 10). Through multiple comparisons tests are observed differences in potassium ratio. These differences reveal that RTRs present median values significantly lower when compared with LKDs group. These results are illustrated in the graphs of Figure 38. No significant differences were observed in ratio saliva/serum of chloride, calcium, sodium, magnesium, iron and phosphate among the studied groups (Table 10).

No significant differences were observed in urea ratio among the studied groups (Table 10). Statistically significant differences were observed in uric acid ratio among RTRs, HD and LKDs groups (Table 10). Through multiple comparisons tests are observed differences in uric acid ratio. These differences reveal that RTRs

present median values significantly lower when compared with RTRs and LKDs group. These results are illustrated in the graphs of Figure 39.

Statistically significant differences were observed in LDL ratio among RTRs, HD and LKDs groups (Table 10). Through multiple comparisons tests are observed differences in LDL ratio. These differences reveal that HD patients present median values significantly higher when compared with RTRs group. These results are illustrated in the graphs of Figure 40.

Statistically significant differences were observed in creatinine ratio among RTRs, HD and LKDs groups (Table 10). Through multiple comparisons tests are observed differences in creatinine ratio. These differences reveal that RTRs present median values significantly lower when compared with LKDs group. These results are illustrated in the graphs of Figure 41.

No significant differences were observed in ratio saliva/serum of triglycerides, total proteins, CRP, albumin and ALP among the studied groups (Table 10).

Statistically significant differences were observed in AST ratio among RTRs, HD and LKDs groups (Table 10). Through multiple comparisons tests are observed differences in AST ratio. These differences reveal that HD patients present median values significantly higher when compared with RTRs group. These results are illustrated in the graphs of Figure 42.

No significant differences were observed in IgA ratio among the studied groups (Table 10).

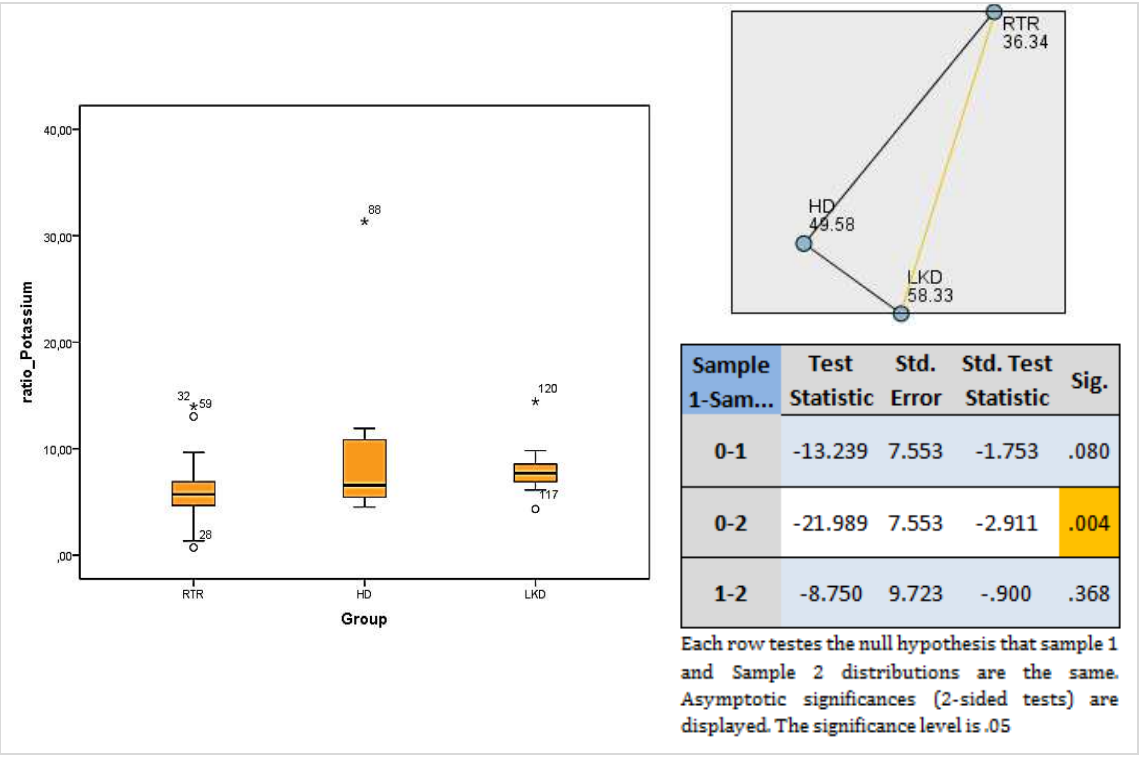
Table 10. Ratio of biochemical parameters in saliva and blood of RTRs, HD and LKDs groups.

Ratio saliva/blood	RTR	HD	LKD	p
Electrolytes				
Potassium	5.72 (5.94±2.36)	6.58 (9.32±7.40)	7.71 (8.02±2.45)	**0.006**
Chloride	0.50 (0.61±0.38)	0.53 (0.58±0.23)	0.63 (0.78±0.32)	0.119

Results

Ratio saliva/blood	RTR	HD	LKD	p
Calcium	0.56 (0.83±0.69)	0.61 (1.05±1.14)	0.74 (1.01±0.82)	0.430
Sodium	0.41 (0.46±0.40)	0.47 (0.36±0.27)	0.37 (0.35±0.24)	0.893
Magnesium	0.10 (0.14±0.16)	0.27 (2.14±5.08)	0.10 (1.01±2.91)	0.444
Iron	0.003 (0.0003±0.0002)	0.0027 (0.0047±0.0064)	0.0011 (0.0011±???)	0.837
Phosphate	5.44 (5.84±2.26)	4.56 (6.31±4.51)	3.98 (6.77±8.27)	0.232
Organic Part				
Urea	1.21 (2.91±12.13)	1.52 (38.76±78.10)	1.69 (3.51±4.5)	0.166
Uric acid	0.62 (0.65±0.39)	0.07 (0.2±0.3)	0.62 (0.69±0.57)	**0.010**
Creatinine	0.08 (0.13±0.12)	0.10 (0.12±0.11)	0.17 (0.23±0.16)	**0.024**
Lipid Profile				
Cholesterol-LDL	0.00 (0.01±0.01)	0.01 (0.04±0.07)	0.00 (0.03±0.06)	**0.009**
Triglycerides	0.07 (0.19±0.29)	0.09 (0.35±0.47)	0.75 (0.96±0.89)	0.056
Proteins				
Total proteins	0.01 (8.38±12.82)	8.63 (16.05±19.20)	14.09 (13.05±14.27)	0.231
C-reactive protein	0.02 (44.17±184.43)	0.01 (0.02±0.02)	0.01 (0.07±0.12)	0.324
Albumin	0.95 (1.46±1.30)	1.34 (1.39±0.78)	0.64 (1.28±1.41)	0.839
Aspartate Aminotransferase	2.08 (2.78±3.92)	3.7 (9.24±10.48)	1.56 (2.63±2.77)	**0.026**
Alkaline phosphatase	0.11 (0.46±1.25)	0.34 (1.34±2.99)	0.10 (0.21±0.28)	0.311
Immunogloblins				
Immunoglobulin A	0.0003 (0.003±0.002)	0.006 (0.007±0.005)	0.0011 (0.0011±0.001)	0.060

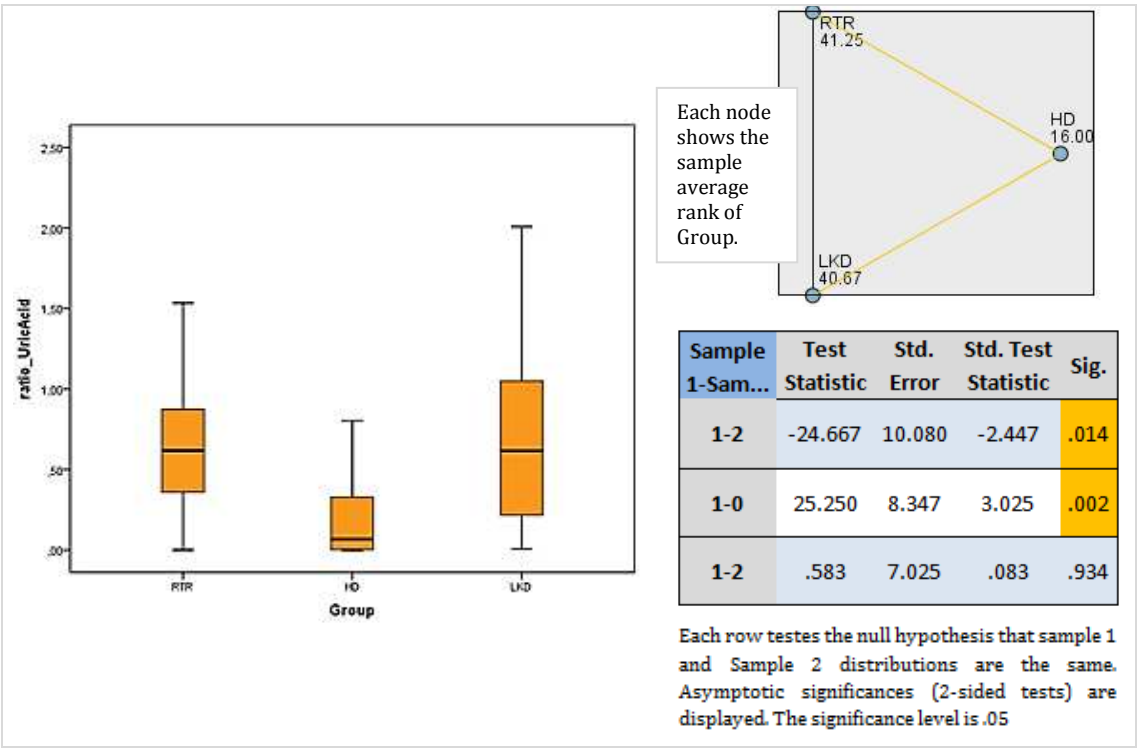
Values are presented as median (mean±standard deviation). Testing of group differences by Kruskall-Wallis test.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
0-1	-13.239	7.553	-1.753	.080
0-2	-21.989	7.553	-2.911	.004
1-2	-8.750	9.723	-.900	.368

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

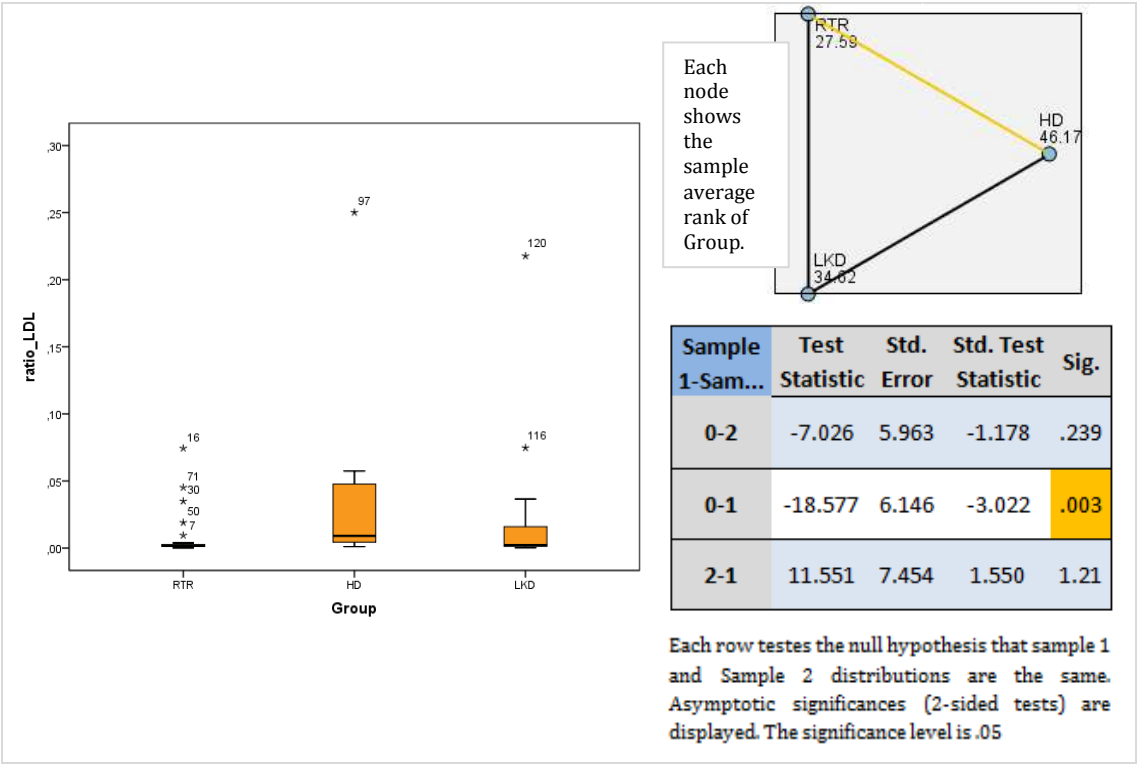
Figure 38. The distribution of potassium ratio saliva/serum of by group. Pairwise comparisons of RTR, HD and LKD groups for potassium ratio saliva/serum.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
1-2	-24.667	10.080	-2.447	.014
1-0	25.250	8.347	3.025	.002
1-2	.583	7.025	.083	.934

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

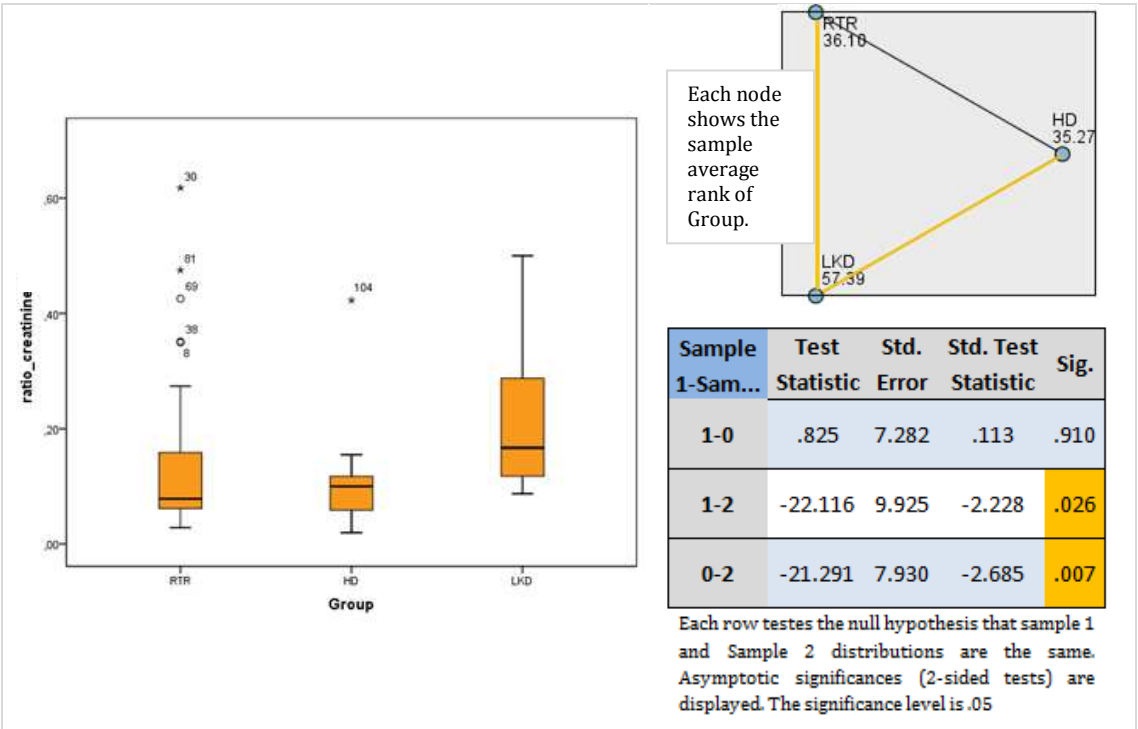
Figure 39. The distribution of uric acid ratio saliva/serum by group. Pairwise comparisons of RTR, HD and LKD groups for uric acid ratio saliva/serum.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
0-2	-7.026	5.963	-1.178	.239
0-1	-18.577	6.146	-3.022	.003
2-1	11.551	7.454	1.550	1.21

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

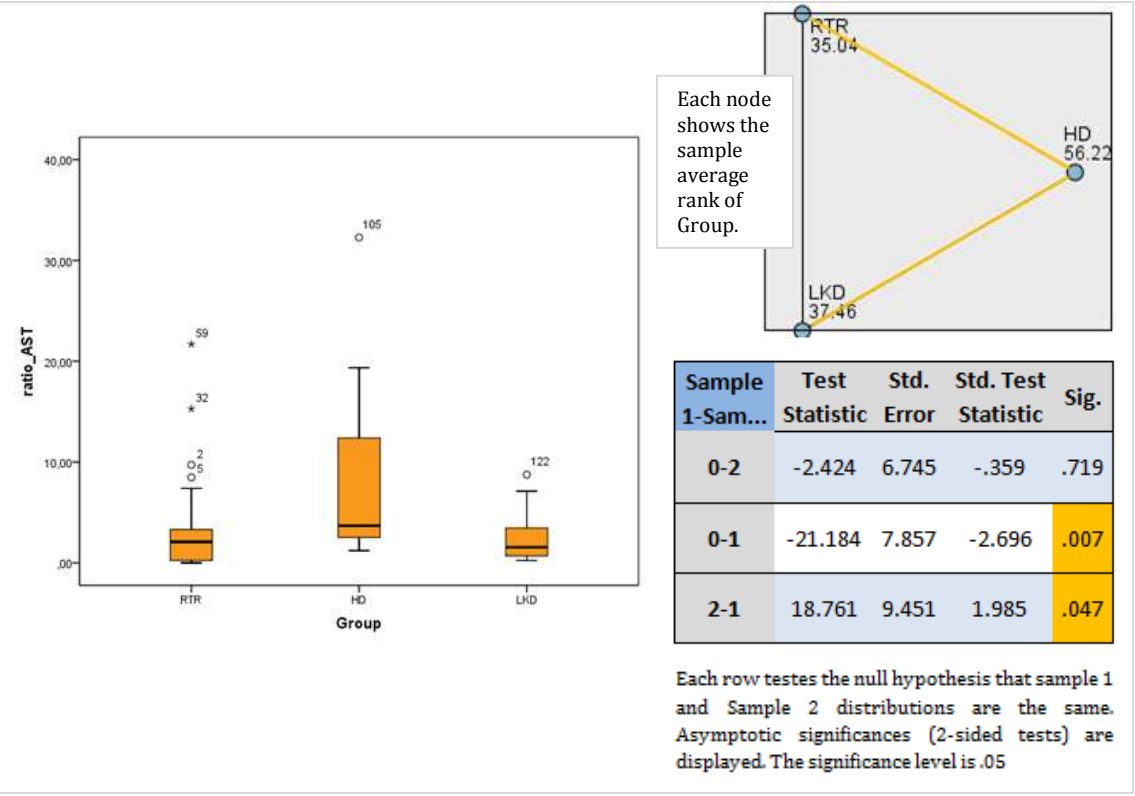
Figure 40. The distribution of LDL ratio saliva/serum by group. Pairwise comparisons of RTR, HD and LKD groups for LDL ratio saliva/serum.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
1-0	.825	7.282	.113	.910
1-2	-22.116	9.925	-2.228	.026
0-2	-21.291	7.930	-2.685	.007

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

Figure 41. The distribution of creatinine ratio saliva/serum by group. Pairwise comparisons of RTR, HD and LKD groups for creatinine ratio saliva/serum.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
0-2	-2.424	6.745	-.359	.719
0-1	-21.184	7.857	-2.696	.007
2-1	18.761	9.451	1.985	.047

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

Figure 42. The distribution of AST ratio saliva/serum by group. Pairwise comparisons of RTR, HD and LKD groups for AST ratio saliva/serum.

The ratio saliva/serum of biochemical parameters in RTRs receiving CsA, Tac and ERL is given in Table 11.

Statistically significant differences were observed in potassium ratio among RTRs receiving CsA, Tac and ERL groups (Table 10). Through multiple comparisons tests are observed differences in potassium ratio. These differences reveal that RTRs receiving Tac present median values significantly higher when compared with RTRs receiving CsA. These results are illustrated in the graphs of Figure 43. No significant differences were observed in ratio saliva/serum of chloride, calcium, sodium, magnesium, iron and phosphate among the studied groups (Table 11).

No significant differences were observed in urea ratio, uric acid ratio, creatinine, cholesterol LDL and triglycerides among the studied groups (Table 11).

Concerning proteins, no significant differences were observed in total proteins ratio among the studied groups (Table 11). Statistically significant differences were observed in CRP ratio among RTRs receiving CsA, Tac and ERL (Table 11).

Through multiple comparisons tests are observed differences in CRP ratio. These differences reveal differences between the three groups. These results are illustrated in the graphs of Figure 44.

Statistically significant differences were observed in AST ratio among RTRs receiving CsA, Tac and ERL (Table 11). Through multiple comparisons tests are observed differences in AST ratio. These differences reveal that RTRs receiving Tac present median values significantly lower when compared with RTRs receiving CsA and ERL. These results are illustrated in the graphs of Figure 45.

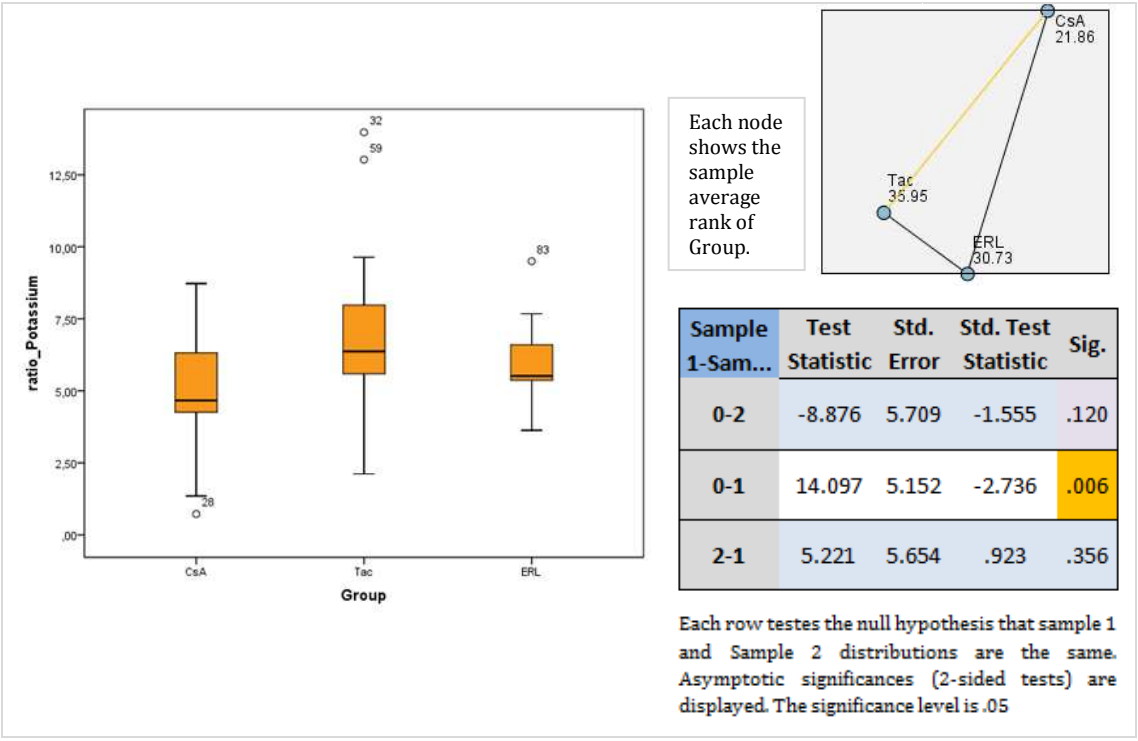
No statistically significant differences were observed in IgA ratio and IgG ratio among RTRs receiving CsA, Tac and ERL (Table 11).

Table 11. Ratio of biochemical parameters in saliva and blood of RTRs receiving CsA, Tac and ERL.

Ratio saliva/blood	CsA	Tac	ERL	p
Electrolytes				
Potassium	4.67 (4.88±2.10)	6.37 (6.92±2.74)	5.51 (6.0±1.37)	**0.022**
Chloride	0.34 (0.59±0.48)	0.57 (0.65±0.35)	0.5 (0.56±0.28)	0.333
Calcium	0.52 (0.96±0.94)	0.56 (0.80±0.59)	0.59 (0.69±0.37)	0.908
Sodium	0.66 (0.63±0.54)	0.24 (0.32±0.35)	0.48 (0.49±0.14)	0.381
Magnesium	0.07 (0.14±0.21)	0.13 (0.17±0.14)	0.10 (0.11±0.06)	0.156
Iron	0.0002 (0.0023±0.0043)	0.0002 (0.0011±0.0020)	0.0003 (0.0005±0.0005)	0.837
Phosphate	4.99 (5.35±2.66)	5.51 (6.41±2.26)	5.70 (5.79±1.74)	0.418
Organic Part				
Urea	1.18 (5.98±21.20)	1.4 (1.48±0.57)	1.21 (1.34±0.55)	0.348
Uric acid	0.45 (0.53±0.41)	0.64 (0.75±0.40)	0.67 (0.63±0.33)	0.164
Creatinine	0.08 (0.1±0.07)	0.1 (0.15±0.14)	0.08 (0.15±0.13)	0.660

Ratio saliva/blood	CsA	Tac	ERL	p
Lipid Profile				
Cholesterol-LDL	0.00 (0.01±0.02)	0.00 (0.00±0.01)	0.00 (0.01±0.02)	0.140
Triglycerides	0.07 (0.23±0.33)	0.08 (0.22±0.32)	0.07 (0.11±0.17)	0.622
Proteins				
Total proteins	0.01 (7.39±13.22)	0.01 (11.71±14.92)	0.01 (5.1±7.85)	0.099
C-reactive protein	0.03 (0.04±0.04)	32.73 (159.62±342.91)	0.01 (0.01±0.01)	**<0.001**
Albumin	1.37 (1.84±1.52)	1.47 (1.49±0.97)	0.38 (0.45±0.21)	0.051
Aspartate Aminotransferase	2.29 (3.57±2.76)	0.00 (2.27±5.45)	2.39 (2.36±0.96)	**0.001**
Alkaline phosphatase	0.13 (0.7±1.79)	0.1 (0.49±1.13)	0.11 (0.15±0.10)	0.914
Immunoglobulins				
Immunoglobulin A	0.0003 (0.0004±0.0003)	0.0002 (0.0003±0.0001)	0.0003 (0.0003±0.0002)	0.430
Immunoglobulin G	0.000 (0.0000±0.0000)	0.000 (0.0000±0.0000)	0.0003 (0.0005±0.0005)	0.158

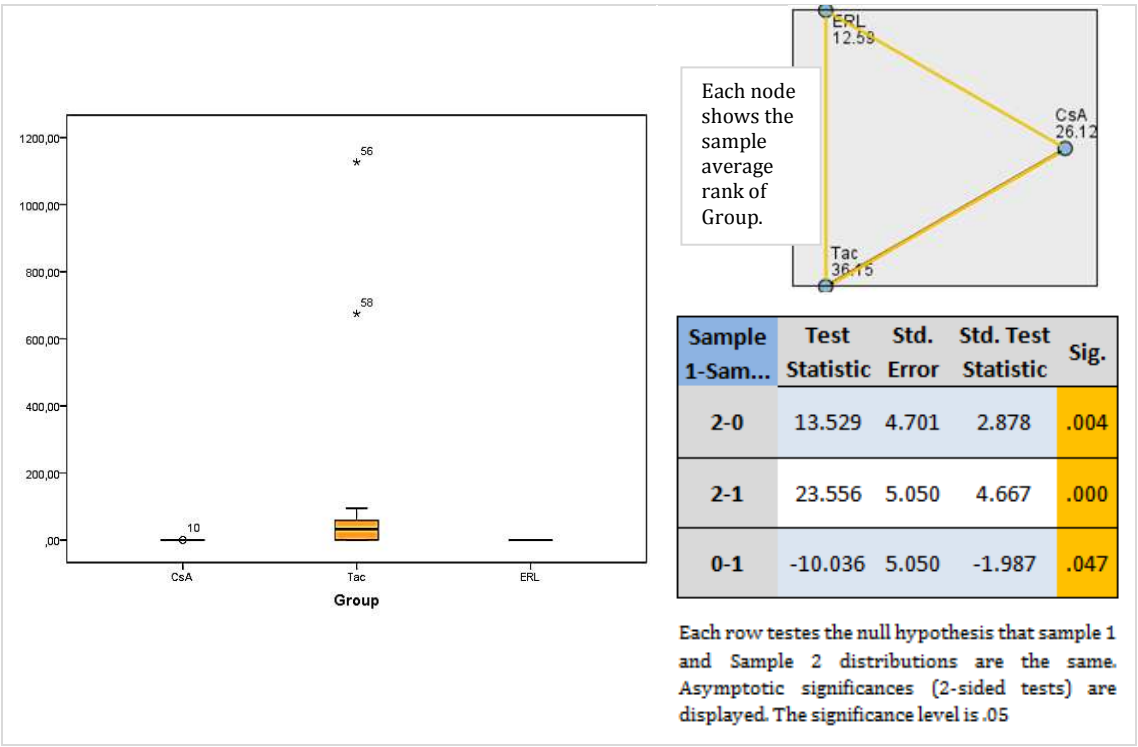
Values are presented as median (mean±standard deviation). Testing of group differences by Kruskall-Wallis test.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
0-2	-8.876	5.709	-1.555	.120
0-1	14.097	5.152	-2.736	.006
2-1	5.221	5.654	.923	.356

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

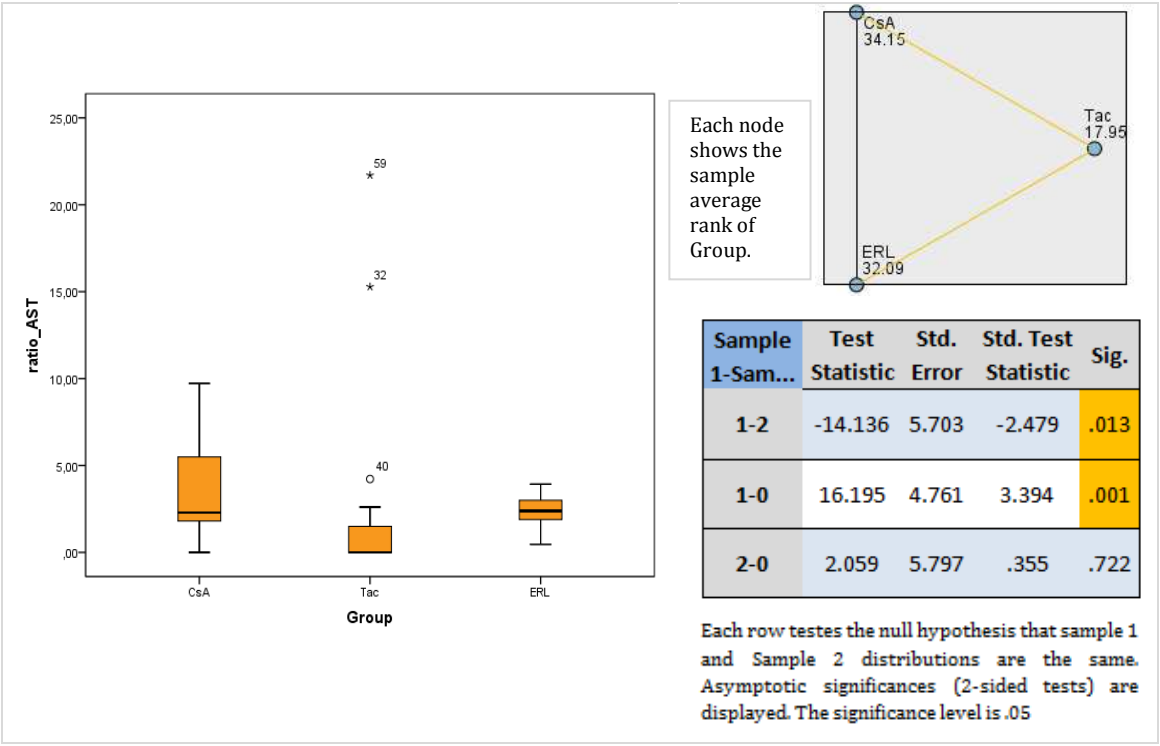
Figure 43. The distribution of potassium ratio saliva/serum by group. Pairwise comparisons of CsA, Tac and ERL groups for potassium ratio saliva/serum.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
2-0	13.529	4.701	2.878	.004
2-1	23.556	5.050	4.667	.000
0-1	-10.036	5.050	-1.987	.047

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

Figure 44. The distribution of CRP ratio saliva/serum by group. Pairwise comparisons of CsA, Tac and ERL groups for CRP ratio saliva/serum.

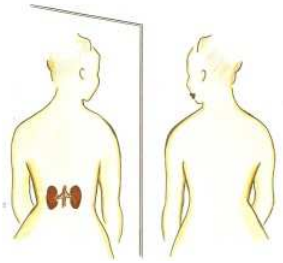


Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.
1-2	-14.136	5.703	-2.479	.013
1-0	16.195	4.761	3.394	.001
2-0	2.059	5.797	.355	.722

Each row testes the null hypothesis that sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05

Figure 45. The distribution of ratio saliva/serum of AST by group. Pairwise comparisons of CsA, Tac and ERL groups for AST ratio saliva/serum.

Chapter IV – Discussion



The main goals of the present study were to evaluate the differences in the oral health status of RTRs receiving CsA, Tac or ERL, and compare it with patients on hemodialysis (pre-transplant) and healthy controls (living kidney donor), evaluate saliva composition and compare it with serum biochemical parameters.

1 Oral health status

1.1 Periodontal inflammation

In the present study, the RTRs receiving ERL presented a lower BOP score in comparison to RTRs receiving CsA and RTRs receiving Tac. Bleeding on probing is universally accepted as a sign of periodontal inflammation that enables the detection of hidden-from-view periodontal inflammation. [134] According to Lindhe and co-workers [86], BOP percentages reflect a summary of the patient's: 1) ability to perform proper plaque control; 2) host response to the bacterial challenge and 3) compliance. The percentage of sites with BOP represents an objective inflammatory parameter, which is used to evaluate the presence of periodontal disease and the risk of disease progression. [86] In addition, Ness and co-workers reported that BOP reflects decreased collagen density, increased blood vessel density and fragility and a reduction of epithelial thickness and integrity. [135] Thin, fragile or even discontinuous pocket epithelium, may serve as an entrance for oral bacteria into the systemic circulation. [135] Furthermore, BOP is characterized by a

dense infiltration of inflammatory cells. [86] These inflammatory cells, which have a key role in the pathogenesis of periodontitis, also may play a role in eliciting a systemic inflammatory response or cross-reactivity. [135] Thus, BOP reflects histological, clinical and bacteriological alterations associated with periodontal disease. [136]

Previous data have shown that CNIs may induce angiogenesis by the production of cytokines whereas mTOR inhibitors may prevent replication of cancer cells and tumor angiogenesis. [137-139] Angiogenesis contributes to inflammation. New blood vessels may contribute to maintain the chronic inflammatory state by transporting inflammatory cells to the site of inflammation and by supplying nutrients and oxygen to the proliferating inflamed tissue. [140] In these conditions, the host inflammatory response in periodontitis leads to soft- and hard-tissue destruction. [141] Therefore, we can hypothesize that RTRs receiving ERL may be more protected for the development of periodontal disease in comparison to RTRs receiving CsA and RTRs receiving Tac, due to the distinct effects of the two immunossupressors in angiogenesis.

If we compare our results with those of other studies that evaluated oral health status in similar populations, our BOP scores may seem low. However, one should mention that our results of BOP scores are presented as median (min-max) because the values did not follow a normal distribution. When we present our results of BOP scores as mean±standard deviation, the values for the CsA, Tac, ERL and LKD groups were 20±22, 15±21, 6±10 and 10±10, respectively, which are similar to those of other studies. [131, 142]

Renal transplant recipients receiving ERL were older than those receiving Tac. This finding is in agreement with previous observations and is related with the fact that Tac is the CNI used in our and other transplant units in RTRs younger than 45 years, whereas in RTRs older than 45 cyclosporine A is the CNI used. [59, 143] Because it is known that the process of ageing may contribute to higher severity of periodontal disease, the finding that RTRs receiving ERL were older and presented less periodontal inflammation further reinforces the view that ERL may be endowed with protective effects on periodontal disease.

The time after transplant was also higher in RTRs receiving ERL than in those receiving CsA and RTRs receiving Tac. This is an expected finding mainly because CsA and Tac are a first step immunossupressive therapy used in the early post-transplant phase whereas ERL is as second step immunossupressor used in RTRs switched from CNIs due to chronic CNI-related nephrotoxicity.

Previous data showed that smoking is a risk factor for periodontal disease. In addition, in smokers the signs and symptoms of both gingivitis and chronic periodontitis, mainly gingival redness and BOP, are masked by the dampening inflammation seen in smokers when compared to non-smokers. [86] In the present study no differences were observed regarding smoking habits, both past and current among the three groups. Hence, we can conclude that the lower BOP scores in RTRs receiving ERL were not affected by smoking as confounder.

Bacterial colonization and growth on supra- and sub-gingival tooth surfaces causes chronic inflammation in periodontal tissues. [144] Periodontitis is an asymptomatic infection that causes local impairment during its lengthened progression, but it may also be associated with increased risk for non-oral infections as well as to pro-inflammatory host responses linked to systemic diseases, namely cardiovascular diseases. [144] Therefore, our results suggest that adequate pre- and post-transplant oral health care may be particularly recommended for RTRs receiving CsA and for those receiving Tac in order to prevent these related co-morbidities.

1.2 Salivary flow rate

We found that unstimulated and stimulated saliva flow rates were significantly lower in HD group than in RTRs group. Given that unstimulated saliva is related to lubricating and antimicrobial functions and stimulated saliva flow rate is related with oral clearance, our results suggest that HD patients could have a source for active infection in oral tissues.

Human saliva lubricates the oral tissues and make possible oral functions such as speaking, eating, and swallowing, but also protects teeth and oral mucosal surfaces

in different ways. [145] The lubricating and antimicrobial functions of saliva are maintained mainly by unstimulated saliva. [145] Stimulated saliva provides a flushing effect and contributes to the clearance of the residues present in the mouth such as non-adherent bacteria, cellular and food debris and noxious agents. [145, 146] If changes in oral health status cause a reduction in salivary flow rate, there would be a drastic alteration in the level of oral cleaning. [146] Our results suggest that HD patients had less oral clearance than RTRs or LKDs, favoring the availability of sugars to the biofilm microorganisms. Therefore, adequate oral health care may be particularly recommended for HD patients in order to prevent infectious foci, because oral pathologies or infections could jeopardize the opportunity to receive a successful kidney transplant.

It has been suggested that restriction of fluid intake is the most important reason for a reduction in saliva flow rate in HD patients. [147-149] Some authors also attribute this finding to a direct salivary gland involvement. [147, 150]

Probably the most important caries-preventive functions of saliva are the flushing and neutralizing effects, commonly referred to as "salivary clearance" or "oral clearance capacity". [32, 145] Although, in the present study HD patients had less oral clearance capacity than RTRs or LKDs, no differences were found in the number of decayed teeth among the three groups. Our findings agree with those of Bayraktar and co-workers. [147]

In our study, visual plaque index revealed an unsatisfactory level of oral hygiene among RTRs and HD groups. In literature, previous authors have reported that many patients receiving renal dialysis are victims of oral neglect. [151, 152] Renal dialysis is time consuming and often individuals do not spend much time taking care of them and may ignore other potential problems. [151, 152] Therefore, comprehensive and periodic oral hygiene instructions are recommended to improve oral self-care behaviors and prevent future dental disease and disease progression. [151] Furthermore, opportunistic focal infections may develop at the site of the new transplant if bacteria-inducing dental treatment is required soon after transplantation. [151, 153]

In literature, the oral health of HD patients when compared with the general population is reported being worse in terms of caries, gingivitis, periodontitis, plaque buildup and general oral health status. [14, 187-190]

However, in several studies a very low and non-significant correlation was obtained, namely for periodontal parameters. [146, 188, 190, 191] Our results did not show differences in terms of caries, gingivitis and periodontitis among HD patients, RTRs and healthy controls, LKDs.

2 Oral fungi characterization

In our study RTRs, HD patients and LKDs presented asymptomatic colonization of fungi in saliva. As expected, Candida *albicans* was the most frequent Candida species identified. Interestingly, non-albicans Candida strains were also identified namely molds. Given that Candida albicans is the most common opportunistic infection agent in the oral cavity, adequate oral health care may be particularly recommended to RTRs, due their immunosssupressive state, in order to prevent systemic candidiasis.

Although certain Candida species are considered to be commensal organisms within the oral cavity, these fungi are also the source of Candida species that cause oral candidiasis and are a potential source to systemic candidiasis. [154, 155]

The prevalence of oral fungi in the present study was similar to the results obtained for the general population, namely 34%. [155]

Patients on hemodialysis are much more likely to die of infectious diseases in comparison to general population due to the higher frequency of infections and also due to the greater severity of infectious in these patients. [4] Although this phenomenon is partly explained by the fact that healthier HD patients are selected for transplantation, there is nevertheless a real susceptibility to infections in HD patients, the etiology of which is multifactorial and includes a state of acquired immunodeficiency, immunocompromise and increased risk of exposure to pathogenic microorganisms. [4]

Renal transplant recipients have increased risk for opportunistic infections that could lead to graft failure. [4] Oral Candida carriage and infection have been reported to be associated with a greater risk for systemic infection in transplant recipients. [110] Prevention of fungal colonization and control of local infection may be of critical importance in avoiding systemic candidiasis. [156]

In our study no differences were found when we compared the prevalence and quantity of fungi identified in saliva among the studied groups. Similar results were reported by Castillo and co-workers. [14] Nevertheless, the fungi colonization in RTRs and HD groups is relevant in what concerns, for example, the immunocompromise condition of these two groups.

Although Candida albicans is the most frequent Candida species in the oral cavity, non-albicans Candida strains play an ever-increasing role as colonization agents. [156] In our results this is enhanced because all groups presented molds, even though incubation conditions have been specific to yeasts.

3 Characterization of saliva composition and its relation with serum

3.1 Electrolytes

Potassium

In the present study HD patients had higher levels of potassium than RTRs or LKDs groups, both in serum and saliva. Furthermore, when comparing HD patients with healthy controls, no differences were found in potassium´s ratio. So, our results suggest that saliva composition in HD patients is influenced by phosphate´s concentration in serum.

Potassium is one of the most abundant ions in the body and is critical for many cell functions. [157] The kidneys regulate potassium excretion [157]. In individuals with CKD, the elimination incapacity of potassium leads to serum concentration rising, hyperkalemia. [158] Hyperkalemia is often asymptomatic and the first

manifestations are: cardiac arrhythmias and muscle paralysis, both life-threatening. [4]

In the oral cavity, the main role of potassium is in saliva´s secretion. [159] The concentration of potassium in saliva rises at low rates of salivary secretion. [160, 161]

The salivary glands' secretion system can actively excrete potassium into the oral cavity. [158] The secretion of potassium in the saliva occurs in two phases. In the first, a transient high rate of potassium secretion occurs when the previously inactive gland is activated by nerve stimulation. Most of the potassium secreted in this phase comes from the intracellular potassium of the gland. In the second phase the output of potassium in the saliva is lower and is derived from serum through the intracellular pool. [159, 160]

Given that potassium levels in saliva rise at low rates of salivary secretion and part of the output of potassium in saliva derives from serum, our results suggest that HD patients had higher levels of potassium in saliva probably due to the lower flow rate of salivary secretion and due to the potassium derived from serum.

Another interesting finding was that the higher levels of salivary potassium in HD patients reflected the higher levels of serum potassium when compared with healthy controls. However, this was not found between RTRs and LKDs.

In our study, when we compared RTRs receiving CsA, Tac and ERL, salivary potassium levels were higher in RTRs receiving Tac than RTRs receiving CsA. In addition, when we compared serum potassium levels, the results were similar. This is shown by the differences in potassium´s ratio between the two groups. This finding adds to our study extra information that allow us to hypothesize that salivary glands activity has determined the higher levels of potassium in RTRs receiving Tac, and not serum.

Phosphate

In the present study salivary phosphate was significantly lower in RTRs receiving CsA than in RTRs receiving Tac and ERL. Given that phosphate in saliva is related with dental structure maintenance, our results suggest that RTRs receiving Tac

and ERL patients are endowed with better conditions to maintain the dental structure in comparison to RTRs receiving CsA.

In our study phosphate´s concentration in saliva was influenced by its concentration in serum for all the studied groups.

High serum phosphate levels are common in patients treated with dialysis. [162] Phosphate retention and hyperphosphatemia contribute to vascular and soft tissues calcification and consequently to the high morbidity and mortality in HD patients. [162-165] Salivary fluid contains electrolytes including phosphate. [163] In our study when comparing HD patients with healthy controls, no differences were found in phosphate´s ratio even though HD patients presented higher serum phosphate concentrations than LKDs and RTRs. So, our results suggest that saliva composition was influenced by phosphate´s concentration in serum.

Recently, Savica and co-workers [163] suggested that the level of salivary phosphate could be used as a marker for the initiation of hyperphosphatemia treatment in chronic kidney disease [163].

From our results we can hypothesize that salivary phosphate can be considered a marker for phosphate´s variations in serum.

In our study when we compared RTRs receiving CsA, Tac and ERL, salivary phosphate was significantly lower in RTRs receiving CsA.

Phosphate concentration in saliva depends on salivary pH and varies in accordance with the salivary flow. [163] The most important biological function of this ion is to maintain the dental structure. Another function is its buffer capacity, relevant only in unstimulated saliva. [146] So, our results suggest that RTRs receiving Tac and ERL had higher phosphate levels in saliva and consequently were endowed with better conditions to maintain the dental structure than RTRs receiving CsA.

3.2 Organic part

Urea

In the present study HD group had higher levels of salivary urea than RTRs or LKDs group. Interestingly, the levels of urea in saliva reflected urea serum levels among the three groups.

Urea is formed in the liver as a major end product of the metabolism of nitrogen-containing substances and is excreted by kidneys. [166] Measurement of urea nitrogen in blood is valuable for diagnosing renal diseases, particularly those associated with a reduction in GFR. [167]

The concentration of urea in saliva is described by Akai and co-workers [167] to reflect urea levels in serum. [167] Our results confirm this finding. No differences were found in urea ratio between HD patients and healthy controls. Saliva composition was influenced by the higher concentration of urea in serum. So, our results suggest that salivary urea can be considered a marker for urea´s variation in serum.

In literature it is described that increased salivary urea levels could induce to calculus formation and contributes to the remineralization of dental enamel. [2, 168] These two processes lead to a lower caries experience and were demonstrated in a study with children. [2, 168] In our study no differences concerning DMFT index were observed among the three groups. Similar results have been reported in literature. [169]

Uric acid

In the present study HD group had lower levels of salivary uric acid than RTRs or LKDs group. Interestingly, the salivary levels of uric acid in HD patients did not reflect the serum levels.

Higher serum uric acid levels are associated with hypertension and diabetes mellitus, two major risk factors for CKD. In addition, this altered biochemical parameter has been shown to be associated with CVD. [170] Cardiovascular disease is the leading cause of morbidity and mortality in HD patients and RTRs.[4]

Uric acid can be found in serum and saliva. In saliva, uric acid appears to be the dominant antioxidant and it is also an important biomarker with clinical importance in monitoring the oxidative stress. [171, 172]

Antioxidants represent one of the defense mechanisms against oxidative stress which are present in all body fluids and tissues, including saliva. [172] The decrease in the levels of these important salivary antioxidants can be considered an important mechanism by which toxic effects of free radicals can initiate oral diseases and destroy the oral cavity homeostasis. [172] So, one can hypothesize that patients undergoing HD are less protected to oxidative damage than both RTRs and LKDs.

Oxidative stress is implicated now in the pathology of several oral diseases, such as periodontitis. [172, 173] In periodontitis, polymorphonuclear leukocytes (PMN) produce a range of antimicrobial factors, such as reactive oxygen species during phagocytosis, this culminates in an increased oxidative damage to gingival tissue, periodontal ligament and alveolar bone. [172, 174] So, one can hypothesize that patients undergoing HD are less protected to oxidative damage that leads to periodontal disease.

In our results HD patients had lower levels of uric acid in saliva and higher levels of uric acid in serum when compared with RTRs and LKDs groups. These were unexpected results considering what has been reported in literature. Further studies are necessary to clarify this finding.

Creatinine

In the present study HD patients had higher levels of salivary creatinine than RTRs and LKDs groups. Interestingly, the salivary levels of creatinine in HD patients reflect the serum levels when compared with healthy controls.

Creatinine is a product of muscle metabolism and is excreted by kidneys. [166] The renal excretion of creatinine is independent of the rate of urine flow except for the cases in which the flow is much less than 0.5 ml. per minute. [166]

In literature the importance of creatinine assessment in saliva, for predicting serum levels, is considered limited. [175] Its value in the qualitative monitoring of renal function may, however, be useful. [175]

In our results, salivary creatinine levels in HD patients have reflected serum levels when compared with healthy controls. So, saliva composition in this group was influenced by the higher creatinine serum levels.

However when comparing creatinine´s ratio between RTRs and healthy controls differences were found. So, in this group salivary creatinine was not an useful marker for serum levels when compared with healthy controls.

3.3 Lipid profile

Low-density lipoproteins, triglycerides

In our study HD group had higher levels of salivary LDL than RTRs group, and saliva LDL levels in HD group reflected the serum levels when comparing with healthy controls. Concerning salivary triglycerides, HD patients had higher levels than RTRs and LKDs groups. In addition, in HD patients triglycerides salivary levels reflected serum levels when compared with healthy controls.

A patient´s lipid profile (total cholesterol, LDL, HDL and triglycerides) is used in diagnosis and treatment of hyper- or dyslipidemia.[4] Chronic kidney disease population is prone to suffer from complex forms of dyslipidemia as well as significant CVD.[4]

Dyslipidemia in HD patients is more frequent than in the general population and is characterized by hypertriglyceridemia and low levels of HDL. [4] However, levels of total cholesterol and LDL are usually normal. [4]

The prevalence of lipid changes in RTRs is very high. Increases in cholesterol and LDL are particularly common. [4] HDL is usually normal, and triglycerides are often increased. [4]

In our results the serum lipid profile in HD patients and RTRs followed the prevalence previous described. In fact, LDL serum levels were higher in RTRs than HD patients and LKDs, and triglycerides serum levels were higher in HD patients than RTRs and LKDs groups.

In our study when we compared RTRs and HD patients with healthy controls, we found that salivary lipid profile reflected serum lipid profile. So, we can hypothesize that salivary lipid profile can be considered a marker for lipid profile variation in serum for HD patients and RTRs.

However, differences in lipid profile ratios were found when we compared RTRs with HD patients.

3.4 Proteins

C-reactive protein

In the present study RTRs receiving Tac presented higher CRP salivary levels than RTRs receiving CsA and ERL.

For RTRs receiving ERL our results have shown an inverse variation between salivary and serum CRP levels, when compared with RTRs receiving CsA and Tac. In addition, in RTRs receiving CsA and Tac salivary CRP levels have reflected serum levels.

C-reactive protein is an acute phase protein synthesized by the liver hepatocytes in response to pro-inflammatory cytokines, and it is found in the blood. [176, 177] Acute-phase proteins are defined as proteins whose serum concentration is altered by at least 25% in response to inflammation. [176, 177] Synthesis of the acute phase protein CRP increases dramatically in response to infection, but mildly elevated levels are also associated with chronic inflammation. [178] In addition, other potential stimuli including smoking, obesity and trauma, may also account for mild increases in CRP. [179, 180]

Our results show that RTRs receiving ERL had higher serum CRP levels than RTRs receiving CsA and RTRs receiving Tac. Given that CRP is a non-specific marker of the acute-phase response and is considered one of the most sensitive markers to assess an individual inflammatory status, our results suggest that RTRs receiving ERL had an ongoing or chronic inflammation.

Unrecognized infections, such as periodontitis, are commonly associated with an enhanced inflammatory response and may induce an acute-phase response, elevating CRP levels. [181] Recent studies of healthy populations have shown that periodontal infections are associated with elevated serum CRP values. [123, 147, 182, 183]

From another perspective, serum CRP in literature is not described as being specifically increased due to periodontal disease, but is increased due to inflammatory conditions caused by many other systemic diseases. [123] Besides that, if serum CRP is used for screening of periodontal disease there is the risk of a crossover effect against a background of systemic diseases. [123] Thus, there are no blood parameters known that exhibit high levels specifically as a result of periodontal disease. [123]

Although CRP is primarily synthesized in the liver, some studies show that human gingiva is able to produce CRP in situ. [181, 184, 185] Because of its non-lipophilic structure and high molecular weight CRP is likely to show limited transfer from blood to saliva. [177, 186]

Our results show that in RTRs receiving ERL serum CRP is not related with its salivary levels. Accordingly, the lack of association between serum and salivary CRP is described by Gomes-Filho. [187]

In addition, our results have shown that RTRs receiving Tac had higher CRP levels in saliva than RTRs receiving CsA and RTRs receiving ERL. So, salivary CRP levels could have reflected a higher inflammatory activity in oral tissues in RTRs receiving Tac. Considering this, we have studied the correlation between salivary CRP levels and inflammatory signs in oral cavity, namely BOP scores. In our results, a positive correlation between salivary CRP levels and BOP was only found for

RTRs receiving Tac (data not shown), this was an unexpected finding considering that both CsA and Tac are pro-inflammatory and for both groups high BOP scores were found. Further studies are necessary to clarify this finding.

Aspartate aminotransferase

In the present study HD patients presented higher salivary AST levels than RTRs and LKDs. Interestingly, the salivary levels of AST in HD patients and RTRs reflected AST serum levels when compared with healthy controls. Given that salivary AST is released from injured and dead cells, these findings suggest that HD patients had more tissue damage in oral cavity than RTRs or LKDs groups.

The enzyme AST is a useful marker following tissue damage. The AST is released from injured and dead cells into extracellular fluid and can be readily assayed in serum, tears and in oral cavity (gingival crevicular fluid and saliva). [188]. In saliva, higher levels of AST indicate increased cell damage in soft tissues, such as periodontium, and metabolic changes in the inflamed gingival tissues. [123, 188]

Accordingly to Nomura and co-workers [123] salivary AST may be a candidate for the screening of periodontitis. In addition, some studies reported a correlation between AST levels in saliva and the progression of periodontal disease. [188-190]

In our results salivary AST levels reflected serum levels in RTRs and HD patients when compared with healthy controls. However, this was not found between RTRs and HD patients.

When we compared RTRs receiving CsA, Tac and ERL, lower levels of salivary AST were found in RTRs receiving Tac.

Lactate dehydrogenase

In the present study RTRs had lower salivary LDH levels than both HD and LKDs groups. The enzyme LDH is a ubiquitous enzyme that plays a significant role in the clinical diagnosis of pathological processes. [123]

The origin of LDH in saliva is being attributed to an extra-salivary gland source and serum, or bacteria. [123] In literature, salivary LDH has been investigated has a biochemical marker candidate for the screening of periodontal disease. [123] Currently, low and normal levels of LDH do not usually indicate a problem.

Alkaline phosphatase

In the present study HD patients had higher levels of salivary ALP than RTRs group. Interestingly, the salivary levels of ALP in HD patients and RTRs reflect the serum levels when compared with healthy controls.

The enzyme ALP is produced by many cells, such as PMN, osteoblasts, macrophages, and fibroblasts within the area of the periodontium and gingival crevice. [191] Its main role is in the normal turnover of periodontal ligament, root cementum and maintenance, and bone homeostasis. [192, 193] ALP was one of the first host enzymes proposed as diagnostic indicators of periodontal status. [194]

Given that ALP is released from PMN during inflammation [195] and from osteoblasts [196] and periodontal ligament fibroblasts [197] during bone formation and periodontal regeneration, respectively [193], our results show that HD patients had higher turnover in these tissues than RTRs.

3.5 Immunoglobulins

Immuglobulin A and Immunoglobulin G

In our study RTRs presented lower IgA levels when compared with HD group. Interestingly, the salivary levels of IgA in HD patients and RTRs reflect the serum levels when compared with healthy controls. Given that immunological protection of mucosal surfaces is mediated primarily by the secretory immune system, particularly IgA in secretions, RTRs were less protected to the adherence and penetration of microorganisms and foreign proteins to oral tissues.

Immunoglobulin A is the second most common serum immunoglobulin and it is produced by plasma cells. Immunoglobulin A is the predominant immunoglobulin secreted in oral mucosal sites and is considered to be a major factor contributing to mucosal health and microbial defense. [198] As IgA passes into the secretions, the epithelial cells produce a protein that is added to it, known as secretory piece. The secretory piece helps IgA to be transported across mucosa and also protects it from degradation in the secretions.

Secretory IgA has a wide range of biological activities against pathogens and is believed to act as an immune barrier to prevent adherence and absorption of microbes and various other antigens to the mucosa. Furthermore, it can neutralize intracellular microbial pathogens within the epithelial cells and facilitate their exclusion into the lumen. [198] Additionally, secretory IgA may play a key role in protection against oral candidiasis.[199]

Serum IgG is responsible for all the immunoglobulin molecules functions. Salivary IgG is an ultrafiltrate of serum IgG, which is modified by the host's general immune response.[200]

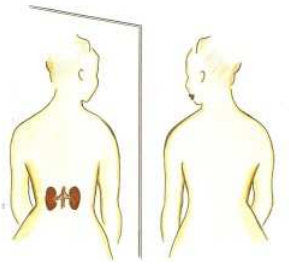
In kidney transplantation immunosuppressive therapy is used to inhibit or prevent activity of the immune system against allograft. However, in RTRs immunosuppressive therapy increases the incidence of infection and malignancy.[55]

In our study RTRs presented lower salivary IgA and IgG levels when compared with HD group. So, RTRs are more prompt to infection in oral tissues than HD patients. Furthermore, salivary levels of IgA in RTRs and HD patients reflected IgA serum levels when compared with healthy controls.

In literature, the protective role of salivary IgA against dental diseases has been a controversial matter [198]. In our study, even though RTRs had lower salivary IgA than HD patients, no differences were found in dental disease among the studied groups. Similar results were found in literature [198] and this could be explained using two perspectives; one is the capacity of immune system to compensate the

decreased of IgA, the other is the minor protective role of salivary IgA against dental disease.

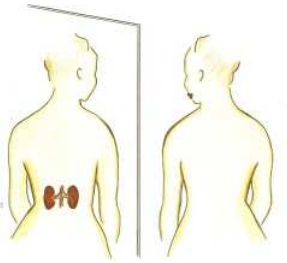
Chapter V – Conclusion



The following conclusions can be drawn from the present study:

- Renal transplant recipients receiving ERL have lower periodontal inflammation and may be better protected for the development of periodontal disease when compared to RTRs receiving calcineurin inhibitors (CsA and Tac).
- The RTRs medicated with Tac differ from those medicated with CsA and ERL in the composition of saliva, namely: potassium, phosphate, C-reactive protein and aspartate aminotransferase.
- Patients on hemodialysis have lower rates of salivary secretion compared with renal transplant recipients.
- Patients on hemodialysis differ from RTRs in the composition of saliva, namely: potassium, urea, creatinine, uric acid, cholesterol-LDL, triglycerides, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, and immunoglobulin A.
- Renal transplant recipients receiving Tac had higher salivary CRP levels this could be considered an indicator of a higher inflammatory activity in oral tissues.

Chapter VI – References



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