Nirmala A. *et al.* / *International Journal of Preclinical & Pharmaceutical Research.* **2013; 4(2): 93-98.**

e-ISSN 2249-7552
Print ISSN 2229-7502

International Journal of
Preclinical & Pharmaceutical Research

Journal homepage: www.preclinicaljournal.com

SALIVA AS A POTENTIAL DIAGNOSTIC TOOL – AN OVERVIEW

A. Nirmala[1], Mazher Sulthana[2], N.S. Jagadeeswari [3], A. S. Smiline Girija[4]

[1]Department of Biotechnology, Aarupadai Veedu Institute of Technology, Paiyanoor, Tamil Nadu, India.
[2]Department of Zoology, Presidency College, Chennai, Tamil Nadu, India.
[3]Department of Biochemistry, Meenakshi Ammal Dental College, Alapakkam Main Road, Maduravoyal, Chennai -95, Tamil Nadu, India.
[4]Department of Microbiology, Meenakshi Ammal Dental College, Alapakkam Main Road, Maduravoyal, Chennai -95, Tamil Nadu, India.

ABSTRACT

Saliva has an old history of study but has physiological importance. Saliva has hundreds of components which help to detect systemic diseases and also has provided biomarkers of health and disease status. Saliva has three major functions: digestion, protection and lubrication. Saliva also functions in maintenance of tooth integrity**.** The antioxidant system also exists in saliva and seems to be of paramount importance. This review examines the diagnostic application of saliva for Diabetes mellitus, renal disease, cardiovascular disease, Infectious diseases etc. As a diagnostic fluid, saliva offers distinctive advantages over serum because it can be collected non-invasively by individuals with modest training. Whole saliva is most frequently used for diagnosis of systemic diseases, since it is readily collected and contains serum constituents. These constituents are derived from the local vasculature of the salivary glands and also reach the oral cavity *via* the flow of gingival fluid. Analysis of saliva may be useful for the diagnosis of hereditary disorders, autoimmune diseases, malignant and infectious diseases, and endocrine disorders, as well as in the assessment of therapeutic levels of drugs and the monitoring of illicit drug use.

Key Words: Saliva, Biological marker, Diabetes, Diagnostic fluid, saliva functions.

INTRODUCTION

The most commonly used laboratory diagnostic procedures involve the analysis of cellular and chemical constituents of blood. Other biological fluids are utilized for the diagnosis of disease, and saliva offers some distinctive advantages. Saliva harbors a wide spectrum of proteins/peptides, nucleic acids, electrolytes, and hormones that originate from multiple local and systemic sources. Although saliva reflects the body's health and well-being, its use as a diagnostic fluid has been hindered, mainly because of our lack of understanding of the biomolecules present in saliva and their relevance to disease etiology, combined with the lack of high-sensitivity detection systems. No special equipment is needed for collection of the fluid.

Corresponding Author

A.Nirmala
Email: nimmi_aruna@yahoo.com

Diagnosis of disease *via* the analysis of saliva is potentially valuable for children, Physically challenged case and older adults, since collection of the fluid is associated with fewer compliance problems as compared with the collection of blood. Further, analysis of saliva may provide a cost-effective approach for the screening of large populations. Saliva can be considered as gland-specific saliva and whole saliva. Gland-specific saliva can be collected directly from individual salivary glands: parotid, submandibular, sublingual, and minor salivary glands. Secretions from both the submandibular and sublingual salivary glands enter the oral cavity through Wharton's duct, and thus separate collection of saliva from each of these two glands is difficult [1]. The collection and evaluation of the secretions from the individual salivary glands are primarily useful for the detection of gland-specific pathology, *i.e.*, infection and obstruction. In this review we highlight the diagnostic potential of saliva for use in detection of disease like diabetes, cardiovascular disease, infectious diseases & renal disease etc.

SALIVA PRODUCTION AND SECRETION

In general, healthy adults produce 500–1500 mL of saliva per day, at a rate of approximately 0.5 mL/min [2], but several physiological and pathological conditions can modify saliva production quantitatively and qualitatively. Smell and taste stimulate saliva production and secretion, as do chewing, psychological and hormonal status, drugs, age, hereditary influences, oral hygiene, and physical exercise [3]. It is important to have sufficient amounts of salivary secretions to maintain good oral hygiene.

BUFFERING CAPACITY OF SALIVA

Saliva acts as a buffering system. The buffering effect of saliva is largely due to bicarbonate/carbonate ions, and to a lesser extent to phosphate-ions and proteins present in saliva, neutralizing acids ingested or produced by microorganisms in the mouth [4-5]. The buffer activity is usually assessed immediately after collection of saliva sample. Several studies indicate that buffer capacity may increase after storage at room temperature. Generally, the accuracy of pH measurements depends on the method of saliva collection and on the time interval between collection and analysis [6].

SALIVA AS A SPECIMEN

The idea of using saliva in diagnosis was made in the second half of the 20th century [7]. Saliva is an accessible fluid that can easily be collected by the patient. Advantages of saliva testing sample are easy and non invasive collection procedure that is neither painful nor traumatic. Saliva is reliable for early detection of certain diseases and monitoring the disease course in the conjunction with treatment and detection of addictive drugs [8,9]. Blood collection is more expensive because it requires the help of a trained technician, it may evoke ethical issue in special populations (infants or elderly persons), it is inconvenient for the patient because samples must be collected in the clinic, and it can be traumatic and stress inducing, especially when repeated samples must be taken [10].

SALIVA COLLECTION

There are numerous methods available for saliva collection, including harvesting whole saliva, individual gland saliva and stimulated or unstimulated saliva. Saliva samples were collected after patients had received their routine check-up. With the patients seated, the saliva was collected over a 5-min period with instructions to allow saliva to pool in the bottom of the mouth and drain to a collection tube when necessary. Subjects were asked not to swallow any saliva for the duration of the collection to allow the calculation of salivary flow rates. At the end of the collection period, saliva volume was measured, the tube sealed and then frozen in dry ice until taken back to the laboratory for processing. Prior to analysis, the saliva was placed into Salivette (Sarstedt, Leicester, U.K.) tubes using a natural cotton swab insert, and centrifuged at 4000*g* for 10 min at 4 ◦C. The supernatant fraction was then aliquotted into storage vials and kept at −80 ◦C until required for analysis. The use of the Salivette tubes was necessary to reduce the high viscosity of the saliva samples that would otherwise prevent accurate pipetting. Preliminary work to compare fresh saliva centrifuged in standard centrifuge tubes with saliva prepared by centrifugation in three types of Salivette (natural cotton insert, citric acid impregnated insert or plain polyester insert) demonstrated that the antioxidant profile from samples prepared using the natural cotton inserts was identical to the native sample. It was inferred from this that the analyts of interest.

SALIVA STORAGE

Saliva specimens, after collection, should preferably be kept on ice, aliquated and frozen as soon as possible to maintain the sample integrity. The refrigeration prevents the degradation of some molecules in saliva and, when necessary, bacterial growth must also be prevented. Moreover, saliva contains bacterial protease enzymes which can degrade several salivary proteins: this can affect protein compound investigation. Storage procedure and time from the collection mainly affect the analysis of the biochemical variables characterized by temperature instability and bacterial growth. Some salivary compounds can have a very short half-life so the sample to be analyzed needs a narrow range of time after collection; other substances can remain stable in saliva for a longer time and may be detected and quantified after a long time [11]. For this reason, the choice of different storage procedure before the analysis depends on the type of molecule, taking into account its stability. We propose, as a general approach to avoid degradation of salivary compounds, the specimens should be stored taking in to account the following outline:

a) Immediately store saliva aliquots without any processing. Specimens can often be stored at room temperature (when analysis is carried out immediately or in 30–90 min from collection), at +4 °C (when analysis is carried out in 3–6 h from collection), at −20 °C and better at −80 °C (when analysis is carried out days to months after collection).

b) Snap-freezing of saliva in liquid nitrogen: Mix each saliva aliquots with an equal volume of 80 % glycerol in H2O, and then dip the sample in liquid nitrogen. This storage procedure aims to inhibit protease activity degrading some salivary protein compounds, such as S-IgA.

c) The enzyme activity present in saliva can be inhibited by adding inhibitors like leupeptin, aprotinin and 4-[2-

aminoethyl] benzenesulfonyl fluoride in the ratio of 10:1 [11].

d) Sodium azide (NaN3) can be added to saliva specimens in attempt to retard bacterial growth. The use of sodium azide does not influence the measurement of salivary markers when serum-based immunoradioassays are modified for saliva, not even if these methods involve separation or extraction steps. But the possible interference of sodiumazide with horseradish peroxidase, a common component of enzyme immunoassays, can be taken into account [12].

DIAGNOSTIC APPLICATIONS OF SALIVA

Salivary diagnostics is an emerging field that has progressed through several important developments in the past decade, including the publication of the human salivary proteome and the infusion of federal funds to integrate nanotechnologies and microfluidic engineering concepts into developing compact point-of-care devices for rapid analysis of this secretion. In this article, we discuss some of these developments and their relevance to the prognosis, diagnosis and management of disease, as an oral target, and cardiovascular disease, as a systemic example for the potential of these bio diagnostics. Our findings suggest that several biomarkers are associated with distinct biological stages of these diseases and demonstrate promise as practical biomarkers in identifying and managing periodontal disease, and acute myocardial infarction. The majority of these studies have progressed through biomarker discovery, with the identified molecules requiring more robust clinical studies to enable substantive validation for disease diagnosis. It is predicted that with continued advances in this field the use of a combination of biomarkers in multiplex panels is likely to yield accurate screening tools for these diagnoses in the near future.

Diabetes

In diabetic patients, as the consequence of hyperglycemia, glucose metabolic products cause micro vascular changes in blood vessels and basal membranes of cells in salivary glands and oral tissues [13]. All these changes cause easier moving of glucose from blood to saliva and gingival fluid. There is available different data in the literature about the relationship between the concentration of glucose in blood and saliva. Glucose is present in saliva of healthy subjects [14,15], but the mechanism of its secretion is not yet known. Proposed paracelullar and intracellular secretion pathways are still hypothetic that have not been validated yet. In patients with type 1diabetes, there has been proved increased [16, 17,18] or decreased glucose concentration in saliva [19] as compared to the control (healthy) group. In saliva of patients with type 2 diabetes, the glucose concentration is higher than in nondiabetic patients [20, 21, 22]. Lasisi & Fasanmade [23] have shown higher concentration of glucose in saliva of diabetic patients as compared to nondiabetics, regardless of periodontal disease presence. This result has indicated that the concentration of glucose in saliva depends on its concentration in serum. However, some studies have indicated no correlation between glucose concentrations in blood and saliva in diabetic patients [24,25,26] Authors believe that increased presence of glucose in saliva in diabetic patients may favor proliferation of microorganisms and promote their colonization on teeth and *oral mucosa*. The analysis of glucose level in saliva is an attempt to find a noninvasive and painless method for frequent monitoring of blood glucose in diabetic patients.

Cardiovascular disease

Cardiovascular disease is a leading cause of death worldwide. Salivary markers such as amylase have been used for postoperative follow-up in patients who had cardiovascular surgery. Low salivary amylase in preoperative patients with aortic aneurysm is associated with increased mortality [27]. A study by Chatteron *et al*. showed in stress, the increase in heart rate is directly related to increased salivary α amylase level [28]. In a case control study, the patients with acute myocardial infarction showed higher levels of creatine phosphokinase (CPK) in saliva as well as in serum. Unstimulated whole saliva (UWS) CPK concentration correlated significantly with serum CPK level on the first day and on the second day of acute myocardial infarction [29]. Hence, the salivary CPK is used as an alternative to serum CPK for diagnosis and monitoring of myocardial infarction.

Renal disease

We have only few reports to employ saliva as a potential screen for renal disorders. Salivary pH shows an increase due to higher urea concentration in dialysis patients [30]. Salivary creatinine levels show a high sensitivity and specificity for determining the presence of renal disease [31]. Researchers found that saliva can be a good tool for early detection of exposure to lead and cadmium since salivary levels of these elements arise from the diffusible fraction of plasma [32,33].

Hereditary disease

Cystic fibrosis is a genetically transmitted disease of children and young adults, which predominately affects exocrine glands. It produces increased sodium chloride concentration in sweat, chronic obstructive pulmonary disease in lungs, and pancreatic insufficiency. A defective electrolyte transport in epithelial cells and viscous mucus secretions from glands and epithelia characterize this disorder [34]. The abnormal salivary secretions in these patients caused clinicians to explore the usefulness of saliva for its diagnosis. The saliva of cystic fibrosis affected patients contains increased level of calcium and proteins, resulting in the formation of insoluble calcium-

protein complexes [35, 36]. The increase in salivary concentration of sodium, phosphate, chloride, lipid, PGE 2 was also observed [37]. Most of the studies concerning the diagnostic application of saliva for cystic fibrosis are relatively old and saliva is not currently used for the diagnosis of this disorder. For diagnosing celiac disease, IgA and antigliadin antibody detection in saliva show high specificity and low sensitivity, whereas their determination in serum is highly sensitive and less specific [38]. Plasma levels of steroids reflect the total measurements, whereas salivary levels of steroids show only the free and active level. Hence, for monitoring steroids, salivary tests are gaining popularity. 21-hydroxylase deficiency is an inherited disorder of steroid genesis, which leads to congenital adrenal hyperplasia.

Infectious diseases

Helicobacter pylori infection is associated with peptic ulcer disease and chronic gastritis. Polymerase chain reaction (PCR) technique has been proved to be highly sensitive and specific for detecting *H. pylori* DNA in saliva. Studies have shown the higher prevalence of *H. pylori* in saliva than in feces, and the oral-oral route may be an important means of transmission of this infection in developed countries [39]. Specific antibody to *Taenia solium* larvae has been demonstrated in saliva for identification of neurocysticercosis. It was suggested that saliva could be used in epidemiologic studies of this disease [40]. *Mycobacterium tuberculosis* can be detected by PCR in the saliva as the bacterial count is high in acute condition of this disease. Salivary IgA response in newborn infants was found to be a better marker of rotavirus infection, compared to the serum antibody response. Hence, saliva rather than serum, can be used to monitor the immune response to vaccination and infection with rotavirus [41]. Saliva has also been used to screen hepatitis B surface antigen in epidemiological studies. Saliva may also be used for determining immunization and detecting infection with measles, mumps, and rubella [42-44].

Endocrinal disorder

Measurement of salivary hormone levels is of clinical importance if they accurately reflect the serum hormone levels or if a constant correlation exists between salivary and serum hormone levels. The majority of hormones enter saliva by passive diffusion across the acinar cells. The small polar molecules enter saliva by ultrafiltration [44]. The lipid-soluble hormones with lower molecular weights can be detected most reliably in saliva, but protein-bound hormones (such as gonadotropins, prolactin, and thyrotropin) cannot be accurately monitored by means of salivary analysis [45]. Due to their lipid solubility, steroid hormones can be detected in saliva.

Monitoring therapeutic levels of drug

The use of saliva for drug monitoring, and the detection of illicit drugs, has grown remarkably. Cotinine, a metabolite of nicotine, is widely used to assess tobacco use and in studies on the effects of smoking on health [46]. For a drug to appear in saliva, drug molecules in serum must pass through the salivary glands and should enter into the oral cavity. Smaller drug molecules which are lipophilic and non-ionized readily diffuse into the oral cavity from serum. The unbound fraction of the drug in serum is usually available for diffusion, which is also pharmacologically active. This active unbound fraction of drug may represent an advantage of drug monitoring in saliva in comparison with serum, which has both bound and unbound fractions. Currently, saliva can be used to detect the level of lithium, carbamazepine, barbiturates, benzodiazepines, phenytoin, theophylline and cyclosporine, antipyrine, caffeine, cisplatin, diazepam, digoxin, ethosuximide, irinotacan, methadone, methoprolol, oxpernolol, paracetamol, primidone, procainamide, quinine, sulfanilamide, tolbutamide, and for drug abuse such as amphetamine, benzodiazepines, cocaine, ethanol, marijuana, nicotine, opioids, and phencyclidine [47].

DNA tests

DNA in cells present in whole saliva could also be investigated. The traditional source of genomic DNA is blood, but recently saliva has increasingly been investigated as a source of DNA deriving from oral cells. It provides a useful source for biomarker profiling and forensic identification. DNA tests in saliva could also be carried out for the detection of HIV infection, recognizing viral sequences in total salivary DNA amplifying by polymerase chain reaction a relatively constant region of HIV-1 genome. In oral fluid are also detectable by PCR several oral pathogens that may cause periodontitis [48].

CONCLUSION

The saliva matrix is an upcoming area of research for basic and clinical application purposes, with considerable potential for growth and progress. Saliva is a really useful specimen when a qualitative answer is required (for example in toxicology). It is also usable for quantitative measurements of several analytes, particularly when a stable correlation between plasmatic and salivary levels can be achieved. Nevertheless, to date salivary assays are still little used compared with plasma assays, even if it is possible to have a quantitative estimate of hormones and other substances in saliva. In conclusion, saliva is a biological fluid that offers several opportunities in diagnosis, toxicology and in forensic science. Furthermore, many salivary proteins offer great potential in clinical and epidemiological research, in oral as well as in general health studies.

REFERENCES

1. Navazesh M. Methods for collecting saliva. *Ann. N. Y. Acad. Sci.,* 694, 1993, 72–7.
2. Chicharro JL, Lucia A, Perez M, Vaquero AF, Urena R. Saliva composition and exercise. *Sports Med.,* 26(1), 1998, 17–27.
3. Walsh NP, Laing SJ, Oliver SJ, Montague JC, Walters R, Bilzon JLJ. Saliva parameters as potential indices of hydration status during acute dehydration. *Med Sci Sports Exerc*, 2004, 1535–42.
4. Lina E, Petra AM, Van den K, René A.de Wijk, Enno GI, Veerman, Arie VNA, Frits B *et al.*, The effect of saliva composition on texture of semi-solids. *Archives of Oral Biology*, 52, 2007, 518-525.
5. Bardow A, Moe D, Nyvad B. The buffer capacity and buffer systems of human whole saliva measured without loss of CO2. *Arch Oral Biol,* 46, 2000, 12.
6. Mandel ID. A contemporary view of salivary research. *Crit Rev Oral Biol Med,* 4, 1993, 599.
7. Richard P, Jiri S, Jana V, Edgar F, Petr M, Jindrich P *et al.*, Saliva as a diagnostic medium. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*, 153(2), 2009, 103-10.
8. Fred Voss H. Saliva as a fluid for measurement of estriol levels. *Am J Obstet Gynecol,* 180, 1999, S226-31.
9. Eliaz K. The diagnostic applications of saliva. A review. *Crit Rev Oral Biol Med,* 13(2), 2002, 197-212.
10. AV Nieuw Amerongen, ECI Veerman. Saliva- the defender of the oral cavity. *Oral diseases*, 8, 2002, 12-22.
11. Nurkka A, Obiero J, Kaythy H, Sscott AG. Effects of sample collection and storage methods on antipneumococcal immunoglobulin A in saliva. *Clin Diagn Lab Immunol*, 10(3), 2003, 357–61.
12. Whembolua GL, Granger DA, Singer S, Kivlighan KT, Marquin JA, Bacteria in the oral mucosa and its effect on measurement of cortisol, dehydroepiandrosterone and testosterone in saliva. *Horm Behav*, 49(4), 2006, 478–83.
13. Qureshi A, Qureshi A, Qureshi H, Khan AA,Blood glucose level, salivary pH and oral bacterial count in type 1 diabetic children. *Infect Dis J*, 16, 2007, 45-8.
14. Jurysta C, Bulur N, Oguzhan B, Satman I, Yilmaz TM, Malaisse WJ *et al.*, Salivary glucose concentration and excretion in normal and diabetic subjects. *J Biomed Biotechnol,* 2009, 430426.
15. Soares MSM, Batista-Filho MMV, Pimentel MJ, Passos IA, Chimenos- Kustner E, Determination of salivary glucose in healthy adults. *Med Oral Patol Oral Cir Bucal*, 14, 2009, 510-3.
16. Iqbal S, Kazmi F, Asad S, Mumtaz M, Khan A. Dental caries and diabetes mellitus. *Pakistan Oral Dent J,* 31, 2011, 60-3.
17. Priyadarshini B, Uma R. Biomarkers in saliva of type I diabetes mellitus for diagnostic and prognostic significance. *Am J Biopharm Biochem Life Sci*, 1, 2012, A14,
18. Busato IMS, Ignacio SA, Brancher JA, Moyses ST, Azevedo-Alanis LR, Impact of clinical status and salivary conditions on xerostomia and oral health-related quality of life of adolescents with type 1 diabetes mellitus. *Community Dent Oral Epidemiol*, 40, 2011, 62-9.
19. Gheena S, Chandrasekhar T, Pratibha Ramani, Salivary characteristics of diabetic children. *Braz J Oral Sci*, 10, 2011, 93-7.
20. Aydin S. A comparison of gherlin, glucose, alpha-amylase and protein levels in saliva from diabetics. *J Biochem Mol Biol*, 40, 2007, 29-35.
21. Vasconcelos AC, Soares MM, Almeida PC, Soares TC. Comparative study of theconcentration of salivary and blood glucose in type 2 diabetic patients. *J Oral Sci*, 52, 2010, 293-8.
22. Abikshyeet P, Ramesh V, Oza N, Glucose estimation in the salivary secretion of diabetes mellitus patients. *Diabetes Metab Syndr Obes*, 5, 2012, 149-54.
23. Lasisi TJ, Fasanmade AA. Comparative analysis of salivary glucose and electrolytes in diabetic individuals with periodontitis. *Ann Ibd Pg Med*, 10, 2012, 25-30.
24. Lopez ME, Colloca ME, Paez RG, Schallmach JN, Koss MA, Chervonagura A, Salivary characteristics of diabetic children. *Braz Dent J*, 14, 2003, 26-31.
25. Carda C, Mosquera-Lloreda N, Salom L, Gomez de Ferraris ME, Peydro A, Structural and functional salivary disorders in type 2 diabetic patients. *Med Oral Patol Oral Cir Bucal.*, 11, 2006, E309-E314.
26. Hegde A, Shenoy R, D'Mello P, Smitha A, Tintu A, Manjrekar P. Alternative markers of glycemic status in diabetes mellitus. *Biomedical Research*, 21, 2010, 252-6.
27. Adam DJ, Milne AA, Evans SM, Roulston JE, Lee AJ, Rucklcy CV *et al.*, Serum amylase isoenzymes in patients undergoing operation for ruptured and nonruptured abdominal aortic aneurysm. *J Vasc Surg*, 30, 1999, 229-35.
28. Chatterton RT, Vogelsong KM, Lu YC, Ellman AB, Hudgens GA. Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clin Physiol*, 16, 1996, 433.
29. Mirzaii-Dizgah I, Jafari-Sabet M. Unstimulated whole saliva creatine phosphokinase in acute myocardial infarction. *Oral Dis*, 17, 2011, 597-600.
30. Shasha SM, Aryeh HB, Angel A, Gutman D. Salivary content in hemodialysed patients. *J Oral Med*, 38, 1983, 67-70.
31. Lloyd JE, Broughton A, Selby C. Salivary creatinine assays as a potential screen for renal disease. *Ann Clin Biochem*, 33, 1996, 428-31.

32. Chiappin S, Antonelli G, Gatti R, De Palo EF. Saliva specimen: A new laboratory tool for diagnostic and basic investigation. *Clin Chim Acta*, 383, 2007, 30-40.
33. Gonzalez M, Banderas JKA ,Baez A, Belmont R. Salivary lead and cadmium in young population residing in mexico city. *Toxicol Lett*, 93, 1997, 55-64.
34. Grody WW. Cystic fibrosis: Molecular diagnosis, population screening, and public policy. *Arch Pathol Lab Med*, 123, 1999, 1041-6.
35. Blomfield J, Rush AR, Allars HM, Brown JM. Parotid gland function in children with cystic fibrosis and child control subjects. *Pediatr Res*, 10, 1976, 574-8.
36. Mangos JA, Donnelly WH. Isolated parotid acinar cellsfrom patients with cystic fibrosis. Morphology and composition. *J Dent Res*, 60, 1981, 19-25.
37. Rigas B, Korenberg JR, Merrill WW, Levine L. Prostaglandins E2 and E2 alpha are elevated in saliva of cysticfibrosis patients. *Am J Gastroenterol*, 84, 1989, 1408-12.
38. Rujner J, Socha J, Barra E, Gregorek H, Madalinski K, Wozniewicz B, *et al*. Serum and salivary antigliadin antibodies and serum Ig A anti-endomysium antibodies as a screening test for coeliac disease. *Acta Paediatr*, 85, 1996, 814-7.
39. Li C, Ha T, Ferguson DA Jr, Chi DS, Zhao R, Patel NR, *et al*. A newly developed PCR assay of *H*. pylori in gastricbiopsy, saliva, and feces. Evidence of high prevalence of H.pylori in saliva supports oral transmission. *Dig Dis Sci*, 41, 1996, 2142-9.
40. Feldman M, Plancarte A, Sandoval M, Wilson M, Flisser A. Comparison of two assays (EIA and EITB) and two samples (saliva and serum) for the diagnosis of neurocysticercosis. *Trans R Soc Trop Med Hyg*, 84, 1990, 559-62.
41. Jayashree S, Bhan MK, Kumar R, Raj P, Glass R, Bhandari N,Serum and salivary antibodies as indicators of rotavirus infectionin neonates. J Infect Dis, 158, 1988, 1117-20.
42. Friedman MG. Radioimmunoassay for the detection ofvirus-specific IgA antibodies in saliva. *J Immunol Methods*, 54, 1982, 203-11.
43. Perry KR, Brown DW, Parry JV, Panday S, Pipkin C, Richards A. Detection of measles, mumps, and rubella antibodiesin saliva using antibody capture radioimmunoassay. *J Med Virol*, 40, 1993, 235-40.
44. Quissell DO. Steroid hormone analysis in human saliva. *Ann N Y Acad Sci*, 694, 1993, 143-5.
45. Vining RF, McGinley RA. Hormones in saliva. *Crit Rev Clin Lab Sci*, 23, 1986, 95-146.
46. Istvan JA, Nides MA, Buist AS, Greene P, Voelker H. Salivary cotinine, frequency of cigarette smoking, and body mass index: Findings at baseline in the lung health study. *Am J Epidemiol,* 139, 1994, 628-36.
47. Richard P, Jiri S, Jana V, Edgar F, Petr M, Jindrich P, *et al*. Saliva as a diagnostic medium. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub, 153, 2009, 103-10.
48. Boutaga K, Savelkoul PHM, Winkel EG, VanWinkelhoff AJ. Comparison of subgingival bacterial sampling with oral lavage for detection and quantification of periodontal pathogens by real-time polymerase chain reaction. *J Periodontol*, 78 (1), 2007, 79–86.