Oral manifestations, microbial study and salivary IgA study in asthmatic patients receiving prednisolone

Mushtaq T. Ibraheem, B.D.S. [(1)]
Fawaz D. Al-Aswad, B.D.S., M.Sc., Ph.D. [(2)]

ABSTRACT

Background: Asthma is a disease of the airways characterized by chronic inflammation associated with airway hyper-responsiveness and airway wall remodeling.

Aims of the study: The aims of this study was to determine the prevalence of oral manifestations , identify different microorganism from oral micro flora and determination of salivary IgA and salivary flow rate in asthmatic patients taking different dose of Prednisolone in comparison with control group.

Subjects, materials and methods: The study included 17 patients under treatment with Prednisolone (10-20 mg),15 patients take (20-30 mg) of Prednisolone and other 18 patients take (30 – 40mg) of Prednisolone, and 25 healthy control group (10 male and 15 female).

Results : The most frequent oral manifestations in asthmatic patients on Prednisolone was burning mouth syndrome, then dry mouth, tooth erosion and white coated tongue and decreased in salivary flow rate. High prevalence of *Staphylococcus aureus* , *Staphylococcus epidermidis* , *streptococcus Viridians* and *Candida albicans* in patients with asthma and difference in oral microbial isolation between asthmatic patients take different dose of Prednisolone and healthy control.The level of salivary IgA in asthmatic patients treated with Prednisolone less than healthy control.

Conclusions: The findings of this study show an obvious difference in the prevalence of oral manifestation and some micro-organisms between patients with asthma and healthy control. Decrease of IgA and salivary flow rate in patients with asthma as compared to healthy control.

Key words: Asthma, Prednisolone, oral manifestations, micro-organisms and Salivary IgA. (J Bagh Coll Dentistry 2014; 26(2): 87-93).

INTRODUCTION

Asthma is a syndrome characterized by airflow obstruction that varies markedly, both spontaneously and with treatment. Asthmatics harbor a special type of inflammation in the airways that makes them more responsive than non-asthmatics to a wide range of triggers, leading to excessive narrowing with consequent reduced airflow and symptomatic wheezing and dyspnea. Narrowing of the airways is usually reversible, but in some patients with chronic asthma there may be an element of irreversible airflow obstruction [(1)].

Typical symptoms include recurrent episodes of wheeze, chest tightness, breathlessness and cough. Commonly, asthma is mistaken for a cold or chest infection that is failing to resolve (e.g. after more than 10 days). Classical precipitants include exercise, particularly in cold weather, exposure to airborne allergens or pollutants, and viral upper respiratory tract infections. Wheeze apart, there is often little to find on examination. An inspection for nasal polyps and eczema should be performed [(2)].

The asthmatic patients had more gingival inflammation , periodontal disease and dental caries, on the other hand low salivary secretion rate and salivary total Immunoglobulin A (IgA) this is important in the development of oral candidiasis [(3)].

The presence of asthma precipitating factors and medication used had a considerable effect on the probability of having symptoms of oral manifestations when diseases compared to healthy individuals therefore asthmatic patients reported more symptoms (dry mouth, sore mouth, halitosis, tempromandibular joint (TMJ) disorder and increase dental caries [(4)].

The cornerstone of maintenance therapy in all but mild intermittent asthma is scheduled administration of inhaled corticosteroids. Long-acting and short-acting bronchodilators are added for additional symptomatic control as needed. Leukotriene inhibitors have been shown to be effective adjuncts in maintenance therapy but do not replace corticosteroids. Theophylline preparations may have additional beneficial effects in some patients, but the narrow therapeutic window and modest efficacy of these preparations limit their value [(5)].

Systemic corticosteroids reduce the inflammatory response and hasten the resolution of exacerbations. They should be administered to all patients experiencing an acute severe attack. They can usually be administered orally as Prednisolone, but intravenous hydrocortisone may be given in patients who are vomiting or unable to swallow. Prednisolone therapy (usually administered as a single daily dose in the morning) should be prescribed in the lowest amount necessary to control symptoms. Patients on long-term oral corticosteroids (> 3 months) or

(1) M.Sc. Student, Department of Oral Diagnosis, College of Dentistry, University of Baghdad.
(2) Assistant professor, Department of Oral Diagnosis, College of Dentistry, University of Baghdad.

receiving more than three or four courses per year will be at risk of systemic side effects [2,1].

Corticosteroid administration can be associated with impairment of host immune responses, T lymphocyte, macrophage, and granulocyte function can be impaired leading to increased susceptibility to infection with opportunistic pathogens. Bacterial pathogens including *Staphylococcus aureus* (*S. aureus*), Gram negative (G-ve) organisms [6].

MATERIALS AND METHODS

Sample

Seventeen patients under treatment with Prednisolone (10-20 mg) ,fifteen patients take (20-30 mg) of Prednisolone and other eighteen patients take (30 – 40mg) of Prednisolone, and twenty five healthy control group with no sign and symptom of any systemic disease and age, sex match with patient groups .They were examined from the period (11/2012- 4/ 2013).

Exclusion criteria:

-Newly diagnosed patients not receiving chemotherapy.
- Patients received radiotherapy.
- Relapsed patients on second line treatment.
- Patients with severe periodontal disease.
- Any other systemic disease.

Method of examination

Oral examination

All the patients examined by a single examiner, under standardized conditions; the oral cavity examined in an artificial light by using a mouth mirror. The procedure of examination of oral soft tissue was done in sequence according to directions suggested by the W.H.O. (1987). The oral manifestations were designed according to the following results:

A- Burning mouth sensation: The diagnostic criteria for burning mouth syndrome in this study was: Pain in the mouth present daily and persisting for most of the day, Oral mucosa is of normal appearance, Local and systemic diseases have been exclude [9]

B- Dry mouth: was diagnosed according to the anamnesis below: Does your mouth feel dry? Do you experience any difficulties in chewing dry foods? Do you experience any difficulties in swallowing dry foods? Are you aware of any recent increase in the frequency of liquid intake?

C -White coated tongue (WCT): Appear as white areas adherent to the dorsum of the tongue, upon removal with cotton applicator they revealed an erythematous mucosa [7].

D -Tooth erosion (TE): Irreversible loss of tooth structure due to chemical dissolution by acids not of bacterial origin, erosion is found initially in the enamel and, if unchecked, may proceed to the underlying dentin [8].

Identification of Bacteria

All the bacteria isolated was identified by
- Colony appearance.
- Biochemical characteristics.
- Gram's stain.

Immunological analysis

The level of salivary IgA in saliva was estimated using Demeditec secretary IgA ELISA (DEXK276).

RESULTS

Distribution of studied samples according to the gender

The study sample consist of 50 asthmatic patients of both sex, there were 18(36%) males and 32(64%) females. Asthmatic patients were divided into three groups 17 patients take (10- 20 mg) Prednisolone of both genders, 5(29.4%) were males and 12 (70.6%) were females,15 patients take (20- 30 mg) Prednisolone 7(46.7%) were males and 8 (53.3%) were females and 18 patients take (30- 40 mg) Prednisolone 6(33.3%) were males and 12(66.7%) were females as seen in table (1) . On The other hand The second group is control group similar in respect to age and gender. Ethnic matched with asthmatic group, they were 25 healthy looking individuals, who have no history or clinic evidence of any disease or obvious abnormalities.

Oral manifestation

A- Burning mouth syndrome

The number of patients with asthma take (10-20 mg) of Prednisolone which have burning sensation are 11(64.7%) and in patients with asthma take (20- 30 mg) were 10(66.7%), while the patients take (30-40mg) have13(72.2%)and all these study groups showed highly significant differences with control group and non significant differences between each other, as appear in figure (1).

B- Dry mouth

There were highly significant differences between asthmatic patients and control group and no significant relationship between the study groups as shows in figure (1). Dry mouth was present in 8(47.1%) asthmatic patients on (10- 20 mg), in 9(60%) asthmatic patients on (20- 30 mg) and in 11(61.1%) asthmatic patients on (30- 40 mg), while not present in control group.

C- White coated tongue

Highly significant differences between asthmatic patients and control group and no significant differences between the studied groups were shown. White coated tongue was present in 2(11.8%) asthmatic patients on (10- 20 mg), in 8(53.3%) asthmatic patients take (20- 30 mg) and in 9(50%) asthmatic patients on (30- 40 mg), while not present in control group as showed in figure (1).

D- Teeth erosion

Out of 17 patients suffering from asthma and take (10- 20 mg) of Prednisolone 3(17.6%),were complaining from teeth erosion, while 7(46.7%) in the patients on (20- 30 mg) and patients on (30- 40 mg) have 9(50%) statistically highly significant differences with control group and no significant differences between each other, as appear in figure (1).

Microbiology

Aerobic bacteria: No significant difference has been found between asthmatic patients and control group except for S. epidermidis, Neisseria and streptococcus viridian as shown in figure (2).

Anaerobic bacteria: No significant difference has been found between asthmatic patients and control group regarding anaerobic microorganism as shown in figure (3).

Candida albicans: A significant difference was established between asthmatic patients and control group as shown in figure (4).

Salivary IgA: The IgA in asthmatic patients with different dose of Prednisolone and healthy control individuals with a mean and SD and Minimum and Maximum Values were evident, the lowest value being related to patients treated with (30-40 mg) of Prednisolone (173.44 µg/ml) and the asthmatic patients on (20- 30mg) of Prednisolone (178.25 µg/ml) followed by that scored by the asthmatic patients on (10- 20mg) of Prednisolone (226.51 µg/ml) ,while the mean IgA value for control group (236.29 µg/ml) as appear in table (2) and table (3).

Salivary flow rate : all doses of treatment were compared against the control groups, with the greatest value of Mean at salivary flow rate parameter was in control groups (2.54), then (1.69) in asthmatic patients on (10-20mg) of Prednisolone ,(1.53) in asthmatic patients on (20-30mg) of Prednisolone and finally (1.46) in asthmatic patients on (20-30mg). The lowest value of Standard division was in control groups (0.52), then (0.55) in asthmatic patients on (30-40mg) of Prednisolon, (0.64) in asthmatic patient take (20-30mg) of Prednisolone and finally (0.79) in asthmatic patient take (20-30mg) as appear in table (4) and table (5).

DISCUSSION

Asthma is a chronic inflammatory disorder of the airways. No single histopathology feature is pathognomonic but common findings include inflammatory cell infiltration with eosinophils, neutrophils, and lymphocytes (especially T lymphocytes); goblet cell hyperplasia, sometimes with plugging of small airways with thick mucus; collagen deposition beneath the basement membrane; hypertrophy of bronchial smooth muscle; airway edema; mast cell activation; and denudation of airway epithelium. This airway inflammation underlies disease chronicity and contributes to airway hyper-responsiveness and, airflow limitation [9].

Oral manifestations

The oral manifestations may occasionally occur before the onset of asthma, be present during the disease process or persist even after the disease has resolved, while at other times the oral manifestations are caused by systemic alterations secondary to asthma.

The presence of asthma precipitating factors and medication used had a considerable effect on the probability of having symptoms of oral manifestations [4].

Differences between this study and other studies is not surprising, taking into consideration the lack of the objective criteria, demographic, ethnic, epidemiological factors, all of these make the comparison of this type of the study and others is difficult.

The use of medication other than for asthma was also a significant risk factor for the prevalence of other symptoms of oral diseases. This was more common among asthmatics than controls. The medications used are not known exactly, but the most likely explanation is the co-morbidity of allergic diseases. Asthmatics often tend to use medications for other allergic conditions, such as rhinitis, conjunctivitis, and dermatitis.

The most frequent oral manifestations was BMS in patients with asthma as compared to healthy control this agreed with Laurikainen and Kuusisto [10].This may be explained by the use of corticosteroids may sometimes increase the risk of Candida infections in mouth and the oral candidiasis is usually associated with a burning sensation in the mouth or on the tongue [11].

The poly-pharmacy is a true risk factor for oral dryness. Dry mouth or xerostomia is defined as an overall reduction of salivary output. It is an adverse effect observed with use of corticosteroids [12].

The present study showed that the prevalence of dry mouth is highly in asthma as compared to healthy control group this similar Laurikainen [13]. They were no reports available about the effects of corticosteroids on the function of the salivary glands but in the lungs one of the effects of corticosteroids is decreased mucous secretion [14].

Regarding white coated tongue, the prevalence of these lesions in asthmatic patients were high as compared to healthy control group this got the same result with Fukushima, et al. [15]. Again this may explained by the patients who are treated with corticosteroids show a higher level of salivary glucose than the control group. This higher glucose concentration can also promote growth, proliferation and adhesion of *Candida* to the oral mucosal cells [16].

There were obvious differences between the level of erosion in asthmatic patient than non asthmatic patient

The explanation of the prevalence of dental erosion among asthmatic patients thus reducing the modifying and protective effects of saliva; Bronchodilator drug relax smooth muscle which affect levels lower oesophageal sphincter in addition to bronchus and thereby potentiate gasrooesophageal reflex which result in tooth erosion [17].

Oral microbiology

Many of the normal flora isolated in this study are either pathogenic or opportunistic .Pathogens like *S. aureus* which is potential pathogens, and it is already a cause of many bacterial disease in humans, The present study showed increased in the isolation of *S. aureus* in asthmatic patients as compared to healthy control with statistically non significant relationship. However, *S.aureus* have caused infections in asthmatic patients treated with corticosteroids [18].

The present study showed increase in the isolation of *S.epidermidis* as compared to the healthy control due to continuous use of antibiotics in hospitals [19].

Alpha-hemolytic Streptococcus Viridans illustrated HS relationship as compared with the healthy control and this agreed with klein, et al. [20] that may be partially explained by the immuno- deficiency caused by glucocorticosteroid treatment.

Difference between micro-flora of asthmatic patients as compared to healthy control, is not surprising taking in the consideration host defense mechanism and drug used attributed to these differences. However, G-ve organisms have caused infections in asthmatic patients treated with corticosteroids [21].

Ulrich [22] stated that a systemic proliferation of un-encapsulated *Neisseria* strains may occur in several immunocompromised hosts .

Corynebaoterium is a genus of G+ve, rod-shaped bacteria. They are widely distributed in nature and are mostly innocuous [23].

Corynebaoterium diphtheria strains constitute part of the normal flora, but certain strains once infected by a phage can cause disease, particularly in a compromised (unhealthy) host, up to our knowledge, this study was the first study isolated *corynbacterium diphtheriod* from asthmatic patients, therefore no explanation could be found.

Klebsiella, E-coli and *Proteus* were isolated in small no. from asthmatic patients made them statistically non advisable, therefore no explanation could offer.

The anaerobic G+ve cocci comprise a diverse group of organisms. The majority of those associated with humans were formerly included *Peptostreptococcus* [24]. They are commensal organisms in humans, living predominantly in the mouth. Under immunosuppressed these organisms can become pathogenic, as well as septicemic, harming their host.

Peptostreptococcus can cause brain, liver, breast, and lung abscesses, as well as generalized necrotizing soft tissue infections. They participate in mixed anaerobic infections, a term which is used to describe infections that are caused by multiple bacteria that do not require or may even be harmed by oxygen [25].

Again the isolation is more in asthmatic patients as compared to healthy individuals, this could be explained by the fact that the immunological aspect which reflect on the composition of normal flora by low level of circulating and secreting antibodies that may cross react with pathogens.

The results of C.Albicanus is ignificant as compared with Control group and this result agreed with Fukushima,et al. [15].

This explained by the patients who are treated with corticosteroids show a higher level of salivary glucose than the control group [16].

Salivary IgA

This study showed that asthmatic patients treated with Prednisolone at different doses illustrated that salivary IgA was different between healthy controls and asthmatic patients. The salivary IgA asthmatic patients was significantly lower than that in the control group this similar with Fukushima et al. [15] , they suggested that steroids have the potential to reduce salivary total IgA and that asthmatic patients with lower salivary total IgA tend to suffer oral candidiasis.

On the contrary, Mandel et al. [26] have reported no difference between salivary IgA levels in asthmatic patients they were on corticosteroids and healthy controls, while Blanca et al. [27] disagree with this study found the salivary IgA increased in response to corticosteroids, also the children with asthma have IgA statistically higher as compared with healthy children [28].

Mona et al. [29] stated that the severity of asthma is directly correlated with the concentration of salivary sIgA and also concentration and composition of oral bacterial flora and the low concentration of sIgA is viewed as a compensating reaction, which nevertheless denotes the immaturity of the protection factors in cases of asthma.

REFERANCES

1. Longo DL, Fauci AS, Kasper DL, Bbraunwald E, Hauser SL, Jameson JL, Lozcalzo J. Harrison's: Principles of internal Medicine. 18th ed. McGraw-Hill Co.; 2011.
2. Colledge NR, Walker BR, Hunter JA, (eds). Davidson's principles and practice of medicine. 21st ed. Churchill Livingstone: Elsevier; 2010. p. 670–8.
3. Fukushima C, Matsuse H, Tomari S, Obase Y, Miyazaki Y, Shimoda T, Kohno S. Oral candidiasis associated with inhaled corticosteroid use: comparison of fluticasone and beclomethasone. Ann Allergy, Asthma Immun 2003; 90: 646– 51.
4. Jaber WF. Salivary analysis and oral symptoms in controlled asthmatic patients. 2005; 2(1): 52-68.
5. Goldman L, Schafer AI, Drazen J. Goldman's Cecil medicine. Part 9: Respiratory diseases. 24th ed. Saunders; 2012.
6. Wiest PM, Flanigan T, Salata RA, Shlaes DM, Katzman M, Lederman MM. Serious infectious complications of corticosteroid therapy for COPD. Chest 1989; 95:1180-84.
7. Greenberg, MS, Glick, M, Ship, JA. Burket's Oral Medicine.11th ed. India: BC Decker Inc.; 2008; p. 34.
8. Davenport T. Signs and symptoms of tooth erosion. http://dentistry.about.com/od/toothmouthconditions/a/teetherosionsym.htm. 2007.
9. McPhee SJ, Papadakis MA. Current medical diagnosis and treatment. 50th ed. McGraw-Hill Co.; 2011.
10. Laurikainen K, Kuusisto P. Comparison of the oral health status and salivary flow rate of asthmatic patients with those of non asthmatic adults-results of a pilot study. Allergy 1998; 53: 316-9.
11. Laurikainen K, Hakama M, Haahtela T. Asthma and Oral Health A Clinical and Epidemiological Study 2002.
12. Toogood JH. Complications of topical steroid therapy for asthma Am Rev Respir Dis. 1990; 141: S89–S96.
13. Torres SR, Peixoto CB, Caldas DM, Silva EB, Akiti T, Nucci M, et al. Relationship between salivary flow rates and *Candida* counts in subjects with xerostomia. Oral Surg Oral Med Oral PatholOral Radiol Endod 2002; 93:149–54.
14. Barnes PJ, Pedersen S. Efficacy and safety of inhaled steroids in asthma. Am Rev Respir Dis 1993; 148:S1 – S26.
15. Fukushima C, Matsuse H, Saeki S, et al. Salivary IgA and oral candidiasis in asthmatic patients treated with inhaled corticosteroid. J Asthma 2005; 42:601–604.
16. Khaledh abu-elteen. The influence of dietary carbohydrates on in vitro adherence of four Candida species to human buccal epithelial cells. Microbial Ecology in Health and Disease 2005; 17: 156-62.
17. Barlett DW and Smith BGN. The dental relevance of gastric reflux. Dent Update. 1996; 23(4):205-8.
18. Staples DJ, Geiding DN, Decker JL, Gordon RS. Incidence of infection in systemic lupus erythematosus. Arthritis Rheum 1974; 17:1-10.
19. Blum-Menezes DOJ, Bratfich MC, Padovezeand ML, Moretti. Hospital strain colonization by Staphylococcus epidermidis. Braz J Medical and Biological Res 2009; 42: 294-8.
20. Klein NC, Go CH, Cunha BA. Infections associated with steroid use. Infect. Dis. Clin. North Am. 2001; 15 (2): 423–32, viii.
21. Buchner LH, Schneurson SS. Clinical and laboratory aspects of listeria monocytogenes infection with a report of 10 cases. Am J Med 1968; 45: 904-21.
22. Ulrich V, Heike C. Lutz von Mu¨ller, Donald B, Johannes E, and Matthias F. Bacteremia in an Immuno-compromised patient caused by a Commensally Neisseria meningitides Strain Harboring the Capsule. Null Locus J clinical microbial 2004; 2898–901.
23. Collins MD, Hoyles L, Foster G, Falsen E. Corynebacterium caspium sp. nov., from a Caspian seal (Phoca caspica) . Int J Syst Evol Microbiol 2004; 54 (Pt 3): 925–8.
24. Ezaki T, Kawamura Y, Li N , Li ZY, Zhao L, Shu S. Proposal of the genera Anaerococcus gen. nov., Peptoniphilus gen. nov. and Gallicola gen. nov. for members of the genus Peptostreptococcus. Int J Syst Evol Microbial 2001; 51:1521–8.
25. Mader JT, Calhoun J. Bone, Joint, and Necrotizing Soft Tissue Infections. In: Baron's Medical Microbiology (Baron S et al., eds.) (4th ed.). Univ of Texas Medical Branch. (via NCBI Bookshelf) ISBN. 1996; 0-9631172-1-1
26. Mandel ID, Barr CE, Turgeon L. Longitudinal study of parotid saliva in HIV-1 infection. J Oral Pathol & Med 1992; 21:209–13.
27. Blanca EN, Lizbeth CH, Rodolfo FR, Arturo B, Saul TA, Vicente CR, Juan JISM. Effect of salmeterol and salmeterol plus beclomethasone on saliva flow and IgA in patients with moderate-persistent chronic asthma. Ann Allergy, Asthma, & Immun 2001; 87:420– 3.
28. Rashkova M, Baleva M, Toneva N, Peneva M, Penka P, Koprivarova K. Secretory immunoglogulin A (S-IgA) in the saliva of children with type 1 diabetes, asthma, systemic health and systemic health but wearing removable orthodontic appliances. OHDMBSC 2009; 8(2): 16-24.
29. Olar M, Luca R, Olar V, Effects of oral hygiene upon the quality of breathing in children suffering from bronchial asthma. Intern J Medic Dent 2012; 2 (3):188-94.

Table 1: Frequencies and Percents of the studied groups due to doses and gender variables with comparison significant

Groups	Gender				Total	
	Male		Female			
G.(10 - 20) mg	5	29.4%	12	70.6%	17	100%
G.(20 - 30) mg	7	46.7%	8	53.3%	15	100%
G.(30 - 40) mg	6	33.3%	12	66.7%	18	100%
Total patients	18	36%	32	64%	50	100%
Control	10	40.0%	15	60.0%	25	100%
Total	28	37.3%	47	62.7%	75	100%
C.S. **P-value**	C.C. =0.126 P=0.750 : NS					

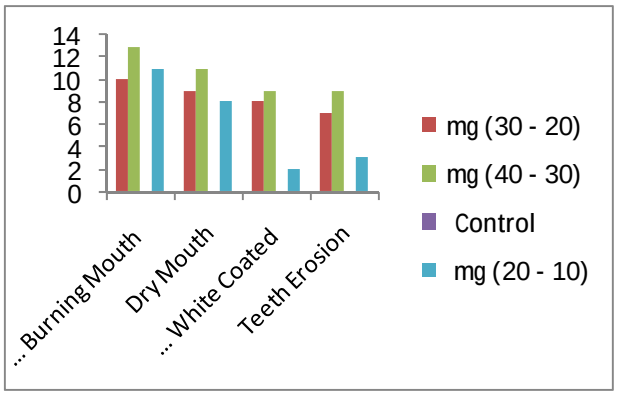

Figure 1: Distribution of the studied oral manifestation parameters based on the different groups of doses

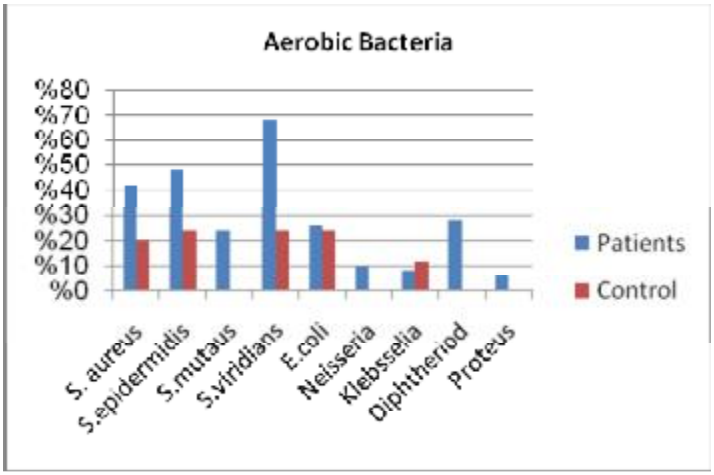

Figure 2: The distribution of "aerobic bacteria" responding based on the different doses.

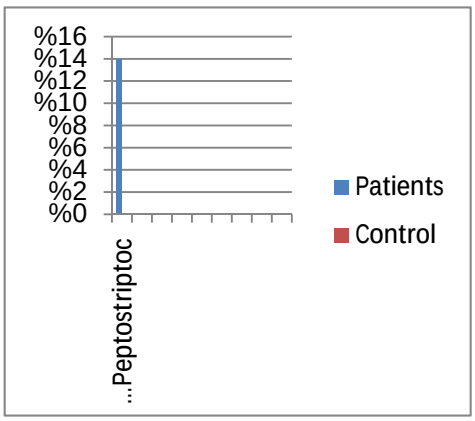

Figure 3: The distribution of anaerobic bacteria responding based on the different doses

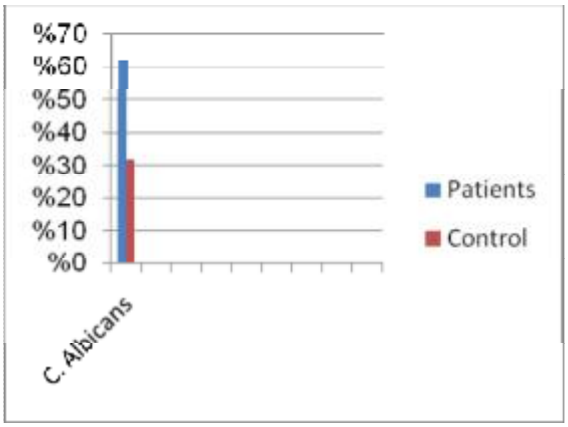

Figure 4: The Distribution of *C Albicans* responding based on the different doses.

Table 2: Summary statistics of salivary IgA parameter at different of the studied groups

Parameter	IgA µg/ml			
Groups	**(10 -20) mg**	**(20- 30) mg**	**(30-40)mg**	**Control**
No.	17	15	18	25
Mean	226.51	178.25	173.44	236.29
Std. Dev.	63.84	63.98	45.41	48.58
Std. Error	15.48	15.48	10.70	9.72
Min.	121.38	121.38	98.98	170.28
Max.	319.72	319.72	250.27	337.13

Table 3: Multiple Comparison among all pairs of IgA parameter according to different treated samples

(I) Group	(J) Group	C.S. (*)
G.(10 - 20) mg	**G.(20 - 30) mg**	S
	G.(30 - 40) mg	HS
	Control	NS
G.(20 - 30) mg	**G.(30 - 40) mg**	NS
	Control	HS
G.(30 - 40) mg	**Control**	HS

HS: Highly Significant at P< 0.01; NS: Non Significant at P>0.05.

Table 4: Summary statistics of salivary flow rate parameter at different of the studied groups

Parameter	Salivary flow rate (ml/5min)			
Groups	**(10 -20) mg**	**(20- 30) mg**	**(30-40)mg**	**Control**
No.	17	15	18	25
Mean	1.69	1.53	1.46	2.54
Std. Dev.	0.79	0.64	0.55	0.52
Std. Error	0.19	0.17	0.13	0.10
Min.	0.50	0.40	0.50	2.40
Max.	3.00	2.50	2.40	3.50

Table 5: Multiple comparison among all pairs of SFR parameter according to different treated samples

Group	Group	C.S.
G.(10 - 20) mg	**G.(20 - 30) mg**	NS
	G.(30 - 40) mg	NS
	Control	HS
G.(20 - 30) mg	**G.(30 - 40) mg**	NS
	Control	HS
G.(30 - 40) mg	**Control**	HS