An Analytical Study of the Bacteria Present in The Saliva Samples of Fasting People and their Comparison with Post-Fasting for the Same People

Shaimaa Hamid Mudher[1], Raida Noori Hamid[2], Fatimah Adulazeez Awad[3]
[1, 2]Department of oral Medicine, College of dentistry, University of Anbar/ Iraq
[3]Department of basic science, College of dentistry, University of Anbar/Iraq
Email: drshom11@yahoo.com

Abstract
This study was carried-out on a 107 student at the period of preparatory school, the student before 10-day from beginning of the experiment not take any antibiotics.Before taking the sample the student wash their nose and mouth thoroughly with water to avoid any contamination to the saliva.This study aimed to investigate the effect of fasting on the amount of bacteria found in the mouth trough show the difference between fasting and non-fasting bacteria in saliva collected from the fasting and non-fasting students.Our study concluded that, the fasting causes reduction of saliva level in the mouth, that causes increasing number of bacteria in the mouth especially cicci, coccobacilli, diplococci and spiral forms of bacteria, that causes a bad odor to the mouth with a harmfull effect on the teeth and degeneration of the soft tissues, cleaning the mouth and thoroughly washing after feed can prevent the growth of these bacteria.
Keywords: bacteria, saliva, fasting people and post- fasting people.

1. Introduction

Saliva is plasma filtration fluid; so, it can be used as a diagnostic fluid rather than serum for biomarkers detection (Yin et al., 2012 and Devanoorkar et al., 2014).
General health is highly related to oral health status, for this reason the huge amount of bacteria on obese patients make them are at a risk for complications of systemic development. The bacteria on the saliva may change the composition of microbiota in the gut because daily swallowing of saliva reach to 1–1.5 liters (Hajishengallis, 2015).
The lining epithelial of the gut has been change by the bacterium P. gingivalis which lead to increase amount of endotoxin (LPS) due to change on the permeability of these epithelium, then systemic inflammation occur due to enterance of the endotoxin to the circulation (Hajishengallis , 2015). Administration of LPS on volunteer caused insulin resistance (Agwunobi et al., 2000).
The main causes for periodontal disease are Treponema denticola, Porphyromonas gingivalis, and Tannerella forsythia (Nachnani et al., 2011).
During fasting there is a decrease on the amount of saliva secretion which effect on self-cleaning of the mouth structures and improper cleaning cause halitosis (Motta et al., 2011).
Halitosis occur because of the ability of these bacteria to produce odorous compounds. The halitosis increase with poor oral hygiene which cause multiplication of bacteria that causes these halitosis. The main bacteria which cause halitosis are proteolytic obligate anaerobes and Gr-negative species, (Tyrrell et al., 2003), they are mainly present on the periodontal pockets and tongue coating bacteria (Awano et al., 2002).
The organic substrate such as mucins, glucose, proteins present in saliva, crevicular fluid, oral soft tissues, and retained debris peptides, all of them are degrade by the bacteria to produce the odorous compound (Aylıkcı and Çolak, 2013).
Gingivitis, periodontitis and caries are caused by bacterial plaque accumulate on oral structure and by food debris with poor oral hygiene (Ship, 2003).
Streptococcus mutans and lactobacilli when present on higher amount in their saliva will cause increased occurrence of dental caries.
In diabetics the bacterial composition of plaque, increased amount of Prevotella nigrescens,Treponema denticola, Streptococcus oralis, Streptococcus sanguinis, and Streptococcus intermedius usually present on supragingival plaque (Hintao et al., 2007).
Oral bacteria when studied by traditional culture teqniques which are low sensitivity, loss of time, expensive, difficult to examine oral bacteria because they not easy to culture or nonculturable. Only the vital bacteria are cultured, so it is important to protect the sample to still viable (Kasap et al., 2009).
This study aims to investigate the effect of fasting on the amount of bacteria found in the mouth trough comparison of the fasting and non-fasting bacteria of saliva collected from the fasting and non-fasting students.

doi.org/10.31838/hiv23.01.112 Received: 04.09.22, Revised: 01.10.22, Accepted: 03.12.22

2. Materials and Methods

1-Patients

A 107 student at the period of preparatory school, the student before 10-day from beginning of the experiment not take any antibiotics.

2-Sample of saliva:

before take the sample the student wash their nose and mouth thoroughly with water to avoid any contamination to the saliva.
We take a sample of saliva from the student during the period of fasting and non-fasting though spatula. After collection of unstimulated of saliva sample from all participant into sterile bottles,expectorate it over 5 minute the dilute it ,it must be done no more than 30 min of the collection of sample . For subsequent analysis it must be archived at−60°C.

3-Rsearch design

The student was fasting for a period of 12-hours and take a breakfast after a fasting period.
We collect the saliva from the student during the fasting and non-fasting period.

4-Bactetriological examination

For bacterial enumeration, samples of human saliva and dental plaque were serially diluted, half-strength thioglycolate medium (USP). The dilution was (0.1 ml), put it in agar media to differentiate the species of oral bacteria.for aerobic bacteria and an aerobic cabinet used Wilkins Chalgren agar. Rogosa agar for total lactobacilli. the plated sample in amedia then immediately transferred to an aerobic cabinet and then the incubation at 37 ± 0.5°C, except of the total aerobe samples, which were incubated in a benchtop incubator (Cole-Parmer, London, United Kingdom) at 37°C for up to 5 days.

3. Statistical Analysis

The statistical analysis was carried-out using t-test for comparison between the fasting and non-fasting groups and Analysis of variance test (ANOVA) for comparison between the male and female in fasting and non-fasting groups. The statistical analysis was carried-out using SPSSPC+-Version 25.

4. Results

1-Number of cocci bacteria among negative and positive cases in female and male during post-fasting and fasting period

Our results observed in Table (1) cleared that, the level of Cocci bacteria differ significantly ($P < 0.01$) among male and female patients and between the fasting and post fasting.
The higher level of cocci bacteria observed in female than male and in fasting than post-fasting individuals. The higher cocci level showed in fasting female 26.59, followed by fasting post-fasting female 23.64 and the lower level observed in post-fasting male 15.77 and post-fasting female 16.87.

Table (1): Number of cocci bacteria among negative and positive cases in female and male during post-fasting and fasting period.

	Group	N	MeanStd. Error	Std. Deviation	Minimum	Maximum
Coccinegative	Post-fasting female	21	16.87±3.66E	13.99	6	60
	Post-fasting male	28	15.77±2.66F	18.49	4	80
	Fasting Female	19	21.46±2.95C	20.79	3	70
	Fasting male	17	19.84±3.29D	24.04	4	90
Coccipositive	Post-fasting female	15	23.86±3.05B	14.17	2	40
	Post-fasting male	30	23.64±3.49B	14.57	2	50
	Fasting Female	37	26.59±5.83A	17.95	4	80
	Fasting male	25	22.16±4.77B	16.43	4	60
Means within the same column of different litters are significantly different at ($P < 0.01$)						

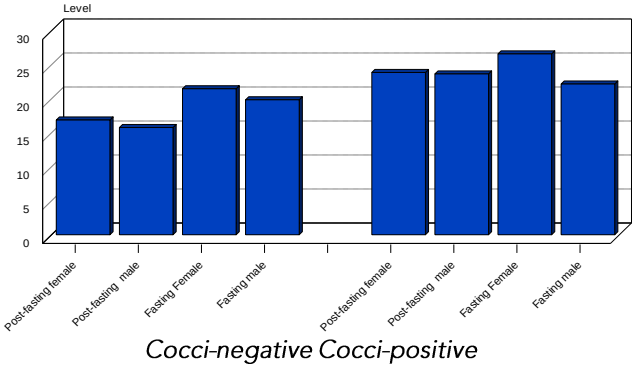

Figure (1): Number of cocci bacteria among negative and positive cases in female and male during post-fasting and fasting period.

2-Number of bacilli bacteria among negative and positive cases in female and male during post-fasting and fasting period.

Our results on the bacilli-bacteria differ significantly ($P < 0.01$) among fasting and post-fasting and among male and female.
The higher bacilli level observed in female than male and post-fasting than fasting individuals.
The higher bacilli level observed in post-fasting female 16.45, followed by post-fasting male as its level reached to 13.22 and the lower level observed in fasting female 7.03, and fasting male 7.03. (Table, 2).

Table (2): Number of bacilli bacteria among negative and positive cases in female and male during post-fasting and fasting period.						
	Group	N	MeanStd. Error	Std. Deviation	Minimum	Maximum
Bacilli-negative	Post-fasting female	33	10.39±1.40 D	8.05	1	30
	Post-fasting male	36	11.72±2.23 C	13.38	2	60
	Fasting Female	34	7.03±1.61H	9.41	1	50
	Fasting male	38	8.95±1.47G	9.06	1	30
Bacilli-pos	Post-fasting female	11	16.45±4.81A	15.95	3	52
	Post-fasting male	23	13.22±3.16B	15.17	2	70
	Fasting Female	45	10.87±2.08E	13.97	2	65
	Fasting male	26	9.19±1.79F	9.14	1	40
Means within the same column of different litters are significantly different at (P < 0.01)						

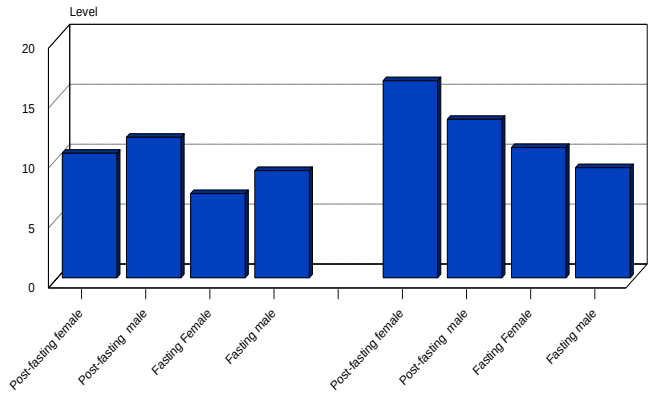

Figure (2): Number of bacilli bacteria among negative and positive cases in female and male during post-fasting and fasting period.

3-Number of diplococcus bacteria among negative and positive cases in female and male during post-fasting and fasting period:

Our results observed in Table (3) cleared that, the diplococcus bacteria level differ significantly (P < 0.01) among male and female and among fasting and post-fasting individuals.

The higher level of diplococcus observed in fasting female as its level were 24.73 followed by post-fasting female 24.73 and the lower level of diplococcus observed in fasting male as the diplococcus level reached to 11.85 folloed by its level in fasting female as its level reached to 14.89.

Table (3): Number of diplococcus bacteria among negative and positive cases in female and male during post-fasting andfating priod.						
	Group	N	MeanStd. Error	Std. Deviation	Minimum	Maximum
Diplo-neg	Post-fasting female	10	24.73±6.52B	13.40	8	50
	Post-fasting male	14	19.00±7.57D	10.66	4	40
	Fasting Female	5	14.89±2.46G	16.61	3	38
	Fasting male	19	11.85±2.12H	23.44	2	80
Diplo-pos	Post-fasting female	11	22.80±4.24C	21.63	2	65
	Post-fasting male	6	15.29±2.85F	18.54	2	50
	Fasting Female	19	26.53±5.38A	10.71	3	40
	Fasting male	20	17.00±7.43E	9.49	4	40
Means within the same column of different litters are significantly different at (P < 0.01)						

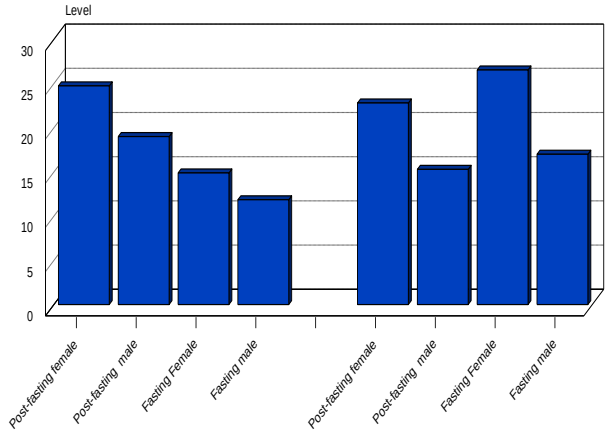

Figure (3): Number of diplococcus bacteria among negative and positive cases in female and male during post-fasting andfating priod.

4-Number of spiral bacteria among negative and positive cases in female and male during post-fasting and fating period.

Our results observed in Table (4) cleared that, the spiral bacteria level differ significantly (P < 0.01) among male and female and among fasting and post-fasting individuals.

The higher level of spiral bacteria observed in fasting male as its level were 20.00 followed by post-fasting male 6.00 and the lower level of spiral bacteria observed in fasting and post-fasting female in negative cases of spiral bacteria as the spiral level reached to 1.00 followed by its level in post-fasting male as its level reached to 1.50.

Table (4): Number of spiral bacteria among negative and positive cases in female and male during post-fasting and fating period.						
	Group	N	MeanStd. Error	Std. Deviation	Minimum	Maximum
Spiral-neg	Post-fasting female	1	1.00F	.	1	1
	Post-fasting male	2	1.50±0.50E	0.71	1	2
	Fasting Female	1	1.00F	.	1	1
	Fasting male	2	1.00±0.01	0.02	1	1
Spiral-pos	Post-fasting female	2	2.50±0.50D	0.71	2	3
	Post-fasting male	2	6.00±0.01B	0.02	6	6
	Fasting Female	3	3.67±2.19C	3.79	1	8
	Fasting male	1	20.00A	.	20	20
Means within the same column of different litters are significantly different at (P < 0.01)						

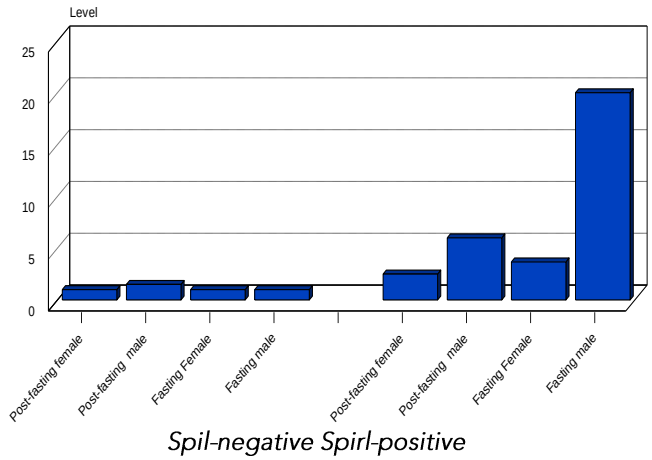

Figure (4): Number of spiral bacteria among negative and positive cases in female and male during post-fasting and fating period.

5. Discussion

Saliva have many functions such as play major role in immunity, protect the oral structure by washing action, buffer agent, play role in re-mineralization, have bacteriocidal and bacteriostatic activities, acidogenic microorganisms clearance (Van Nieuw Amerongen et al., 2004).

Our results on the cocci bacteria among negative and positive cases in female and male during post-fasting and fasting period, cleared that, the higher level of cocci bacteria observed in female than male and in fasting than post-fasting individuals. The higher cocci level showed in fasting female 26.59, followed by fasting post-fasting female 23.64 and the lower level observed in post-fasting male 15.77 and post-fasting female 16.87.

The increasing level of cocci bacteria in fasting than the post-fasting attributed to the overgrowth of the bacteria with reduction of saliva level that occure during the fasting period, with reduction of mucine and lysozyme that prevent the growth of bacteria and act as antibacterial immunity factors.

While our results on the number of bacilli bacteria among negative and positive cases in female and male during post-fasting and fasting period cleared that, the bacilli-bacteria differ significantly (P < 0.01) among fasting and post-fasting and among male and female. The higher bacilli level observed in female than male and post-fasting than fasting individuals. The higher bacilli level observed in post-fasting female 16.45, followed by post-fasting male as its level reached to 13.22 and the lower level observed in fasting female 7.03, and fasting male 7.03.

Meanwhile, our results on the number of diplococcus bacteria among negative and positive cases in female and male during post-fasting and fasting period cleared that, the higher level of diplococcus observed in fasting female as its level were 24.73 followed by post-fasting female 24.73 and the lower level of diplococcus observed in fasting male as the diplococcus level reached to 11.85 followed by its level in fasting female as its level reached to 14.89.

While, our results on the number of spiral bacteria among negative and positive cases in female and male during post-fasting and fating period cleared that, the higher level of spiral bacteria observed in fasting male as its level were 20.00 followed by post-fasting male 6.00 and the lower level of spiral bacteria observed in fasting and post-fasting female in negative cases of spiral bacteria as the spiral level reached to 1.00 followed by its level in post-fasting male as its level reached to 1.50.

The increasing bacterial number in oral cavity and saliva of human attributed to during fasting in oral cavity, 37°C the temperatures ranged between34-37°C m changed between 34 and 37°C (Nodelman et al., 1998), in oral exhalations the humidity may reached to 96% between 91% and 96%, (Zehentbauer et al., 2000). these make suitable environment for growth of pathogens, over 500 species of bacteria are found in oral cavity, (Soder et al., 2000) and the

Our results agreed with those of (Miyazaki et al., 1995) where they reported that, the number of bacterial species, which are found in oral cavity, are over 500 and the most species of them which includes cocci, coccobacilli, spiral and diplococci, (Kharbanda et al., 2003).

These bacteria include especially Gr-negative species and proteolytic obligate anaerobes and they mainly retained in tongue coating and periodontal pockets. (Gurbuz et al., 2010).

Sometime the halitosis occur on people who have no history of periodontal disease and no history of halitosis because of the stagnation of bacteria on the

surface of the tongue (Porter, 2012).
organic substrates have been degrade by bacteria which causes ahramful effect on the mouth. (Davies and Epstein, 2010).
Our study concluded that, the fasting causes reduction of saliva level in the mouth, that causes increasing number of bacteria in the mouth especially cicci, coccobacilli, diplococci and spiral forms of bacteria, that causes a bad adour to he mouth with a harmfull effect on the teeth and degeneration of the soft tissues, cleaning of the mouth and thoroughly washing after feed can prevent the growth of these bacteria.

References

Agwunobi, A. O.; Reid, C.; Maycock, P.; Little, R. A. and Carlson, G. L. "Insulin resistance and substrate utilization in human endotoxemia," The Journal of Clinical Endocrinology and Metabolism, vol. 85, no. 10, pp. 3770–3778, 2000.

Awano S, Gohara K, Kurihara E, Ansai T, Takehara T. The relationship between the presence of periodontopathogenic bacteria in saliva and halitosis. International dental journal. 2002; 52:212–6.

Aylıkcı, B. U and Çolak, H. (2013): Halitosis: From diagnosis to management. J Nat Sci Biol Med. 2013 Jan-Jun; 4(1): 14–23.

Davies A, Epstein JD. Oral complications of cancer and its management. Oxford: Oxford University Press; 2010. pp. 230–240.

Devanoorkar, A.; Kathariya, R.; Guttiganur, N.; Gopalakrishnan, D. and Bagchi, P. "Resistin: a potential biomarker for periodontitis influenced diabetes mellitus and diabetes induced periodontitis," Disease Markers, vol. 2014, Article ID 930206, 7 pages, 2014.

Gurbuz T, Tan H. Oral health status in epileptic children. Pediatrics international: Official journal of the Japan Pediatric Society. 2010; 52:279–83.

Hajishengallis, G. "Periodontitis: from microbial immune subversion to systemic inflammation," Nature Reviews Immunology, vol. 15, no. 1, pp. 30–44, 2015.

Hintao J, Teanpaisan R, Chongsuvivatwong V, Ratarasan C, Dahlen G. 2007. The microbiological profiles of saliva, supragingival and subgingival plaque and dental caries in adults with and without type 2 diabetes mellitus. Oral Microbiol. Immunol. 22:175–181.

Kasap E, Zeybel M, Yüceyar H. Halitosis. Güncel Gastroenteroloji 2009. 2009; 13:72–6.

Kharbanda OP, Sidhu SS, Sundaram K, Shukla DK. Oral habits in school going children of Delhi: A prevalence study. Journal of the Indian Society of Pedodontics and Preventive Dentistry. 2003; 21:120–4.

Miyazaki H, Sakao S, Katoh Y, Takehara T. Correlation between volatile sulphur compounds and certain oral health measurements in the general population. Journal of periodontology. 1995; 66:679–84.

Motta LJ, Bachiega JC, Guedes CC, Laranja LT, Bussadori SK. Association between halitosis and mouth breathing in children. Clinics. 2011; 66:939–42.

Nachnani S. Oral malodor: Causes, assessment, and treatment. (26-28, 30-21;).Compend Contin Educ Dent. 2011; 32:22–24. quiz 32, 34.

Nodelman V, Ben-Jebria A, Ultman JS. Fast-responding thermionic chlorine analyzer for respiratory applications. Review of Scientific Instruments. 1998; 69:3978–83.

Porter SR. Diet and halitosis. Current opinion in clinical nutrition and metabolic care. 2011; 14:463–68.

Ship JA. 2003. Diabetes and oral health: an overview. J. Am. Dent. Assoc. 134(Spec No):4S–10S

Soder B, Johansson B, Soder PO. The relation between foetor ex ore, oral hygiene and periodontal disease. Swedish dental journal. 2000; 24:73–82.

Takeuchi H, Machigashira M, Yamashita D, et al. The association of periodontal disease with oral malodour in a Japanese population. Oral diseases. 2010; 16:702–6.

Tangerman A, Winkel EG. Intra- and extra-oral halitosis: Finding of a new form of extra-oral blood-borne halitosis caused by dimethyl sulphide. Journal of clinical periodontology. 2007; 34:748–55.

Tyrrell KL, Citron DM, Warren YA, Nachnani S, Goldstein EJ. Anaerobic bacteria cultured from the tongue dorsum of subjects with oral malodor. Anaerobe. 2003; 9:243–46.

Van Nieuw Amerongen A., Bolscher J. G., Veerman E. C. (2004) Salivary proteins: protective and diagnostic value in cariology? Caries Res. 38, 247–253.

Yin, J.; Gao, H.; Yang, J.; Xu, L. and Li, M. "Measurement of salivary resistin level in patients with type 2 diabetes," International Journal of Endocrinology, vol. 2012, Article ID 359724, 5 pages, 2012.

Zehentbauer G, Krick T, Reineccius GA. Use of humidified air in optimizing APCI-MS response in breath analysis. Journal of agricultural and food chemistry. 2000; 48:5389–95.

Zehentbauer G, Krick T, Reineccius GA. Use of humidified air in optimizing APCI-MS response in breath analysis. Journal of agricultural and food chemistry. 2000; 48:5389–95.