Original Article

The Oral Adverse Effects of Isotretinoin Treatment in Acne Vulgaris Patients: A Prospective, Case–control Study

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Abstract

Background: Isotretinoin is the most effective therapy to treat severe acne vulgaris and its systemic adverse effects have been well documented, but little is known on dental side effects over the course of treatment. **Objectives:** This prospective case-control study aimed to evaluate the oral adverse effects of isotretinoin in Turkish patients with acne vulgaris; compare oral conditions between patients and normal controls; and investigate the association between salivary parameters and International Caries Detection and Assessment System (ICDAS) scores. **Materials and Methods:** For 6 months, the patients (n = 45) received isotretinoin daily (0.5 mg/kg). The age-matched untreated controls (n = 45) were patients without acne. Both groups were examined before the study and at 6 months for salivary flow, buffer capacity, microbiologic tests, and caries status (based on the ICDAS). Salivary parameters and ICDAS scores were analyzed by Spearman's rank correlations. Data were statistically analyzed by the Mann–Whitney U test, Wilcoxon signed rank tests, and McNemar's Chi-square tests ($P < 0.05$). **Results:** Twenty-two isotretinoin-treated patients and 18 controls completed the study. At baseline, the groups were not significantly different in the evaluated parameters ($P > 0.05$). At 6 months in the isotretinoin-treated group, salivary flow and buffer capacity significantly decreased, and the ICDAS scores significantly increased ($P < 0.05$). The changes in these criteria from baseline were insignificant in the controls ($P > 0.05$). Intraoral pathogen counts were not significantly different between the groups, compared to baseline ($P > 0.05$). Stimulated salivary parameters in both groups were not correlated significantly with the ICDAS scores. **Conclusions:** Isotretinoin significantly affected salivary flow, buffer capacity, caries lesion activity scores for 6 months. However, salivary parameters and caries lesion activity scores had no significant correlations.

Keywords: *Acne vulgaris, dental caries, International Caries Detection and Assessment System, isotretinoin, salivary flow*

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Introduction

Acne vulgaris affects more than 80% of adolescents and is the most frequently encountered skin disease of teenagers.[1] There is no ideal treatment for acne, although a suitable regimen can reduce lesions in most patients.[2] In most treatment regimens, oral and/or topical antibiotics,[3,4] and oral isotretinoin are recommended. Retinoids are well-known therapeutic agents that can cause dryness of the mouth and oral mucosa.[5] Previous clinical studies demonstrate that isotretinoin adversely affects salivary flow and buffer capacity.[6-8]

Dental caries is a very prevalent, but preventable, chronic oral disease of humankind that is characterized by progressive tooth demineralization due to the action

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of bacterial acid metabolism; saliva has a significant role in the development and/or prevention of the disease.[9-11] Protection of the oral cavity requires a sufficient amount of saliva and depends on saliva properties such as flow, pH, and buffer capacity.[12] Medications that alter salivary flow are a recognized risk factor for dental caries.[5,13]

The decayed-missed-filled teeth (DMFT) index is a classic epidemiologic index for recording the incidence and prevalence of caries.[14] In this index, missed and filled teeth are not always indicative of past caries experience; hence, the index overestimates the caries experience of a population.[14,15] In recent years, the International Caries Detection and Assessment System (ICDAS) criteria have been recommended for use in epidemiological studies to overcome the limitation of the DMFT index.[16,17]

However, only limited research exists in the literature on the adverse dental effects of isotretinoin on salivary flow,[7,8] buffer capacity,[8] pathogen microorganism counts, and DMFT scores in patients with acne vulgaris.[8] But, there was no study found investigating association between salivary parameters and ICDAS scores as well as the effect of age, salivary parameters, and ICDAS scores on caries occurrence. Therefore, the aim of this study was to examine the adverse oral effects of isotretinoin in a group of adolescent patients with acne vulgaris in comparison with a control group without acne vulgaris by using salivary flow, buffer capacity, microbiological tests, and ICDAS-II criteria. In this study, the null hypothesis was that during a 6-month period, (1) there would be no significant oral condition changes between the isotretinoin-treated patients and the control group patients and (2) there would be no association between salivary parameters (i.e., salivary flow, buffer capacity) and the ICDAS scores.

Materials and Methods

Protocol

This clinical prospective case–control study was performed in Istanbul, Turkey by two dermatologists and two dentists who worked in collaboration. The test group patients were recruited from private and public medical hospital Dermatology Departments after seeking therapy for acne vulgaris. The control group comprised dental clinic patients recruited from Istanbul University (Faculty of Dentistry in the Department of Operative Dentistry; Istanbul, Turkey); they had attended the clinics for routine dental care. Before the study, the dental examiners gave all dermatologists verbal and written descriptions of the protocol about this clinical case–control study.[8] In accordance with the protocol, the dermatologists were advised to collect the patients' informed consent forms and to inform dentists to examine the included patients before initiating isotretinoin therapy. Determination of the sample size for this investigation was analyzed by power analysis at a power of 80% with a 95% confidence level and significance level set at 0.05. Power analysis was performed in accordance with a previous investigation which allowed us to estimate the sample sizes.[18] An equal size of test and control patients were formed, which ensured 45 patients for each group. Both test and control group patients were selected and evaluated for this study from June 2013 to June 2014. The study conformed to good clinical practice guidelines, and the research protocol was approved by the Ethical Committee at Istanbul University Faculty of Medicine, Istanbul, Turkey (Project no: 2013/370).

Patient selection

For the test group, dermatologists included in the study 14 male and 31 female patients who were unresponsive to conventional acne vulgaris therapy had requested treatment for acne vulgaris at the Department of Dermatology in Medical Park Hospital (a private hospital) and Okmeydani Training and Research Hospital (a public hospital) in Istanbul, Turkey, were 18–25-year-old (mean age: 22 years) and in good general health. For the control group, the dentists randomly included in the study 12 male and 33 female patients who had requested routine dental treatment at the Istanbul University at the Faculty of Dentistry in the Department of Operative Dentistry were 18–24-year-old (mean age: 21 years) and in good general health. Patients were not admitted to the study if any of the following criteria were detected: (1) Additional dermatological disorders, (2) orthodontic treatment, (3) the presence of a systemic disease that modified salivary flow, (4) the use of medications that cause hyposalivation, (5) pregnant or lactating patients, (6) residing outside Istanbul city, and (7) insufficient address for follow-up.

Treatment regimen

Potential test group patients with acne vulgaris who had indications for isotretinoin treatment were informed by their dermatologist about the study. The patients who agreed to participate in the study received a daily dose of isotretinoin (0.5 mg/kg) for 6 months.[8] Potential control group patients, whose mean age was similar to that of the test group patients, were also informed about the study by their dentist. They then agreed or refused to participate. Each study participant in both groups underwent two appointments. The first before the beginning of the study and the second at the end of the medication treatment period at 6 months. During the study period, all patients were advised by their dentist to follow food intake and oral hygiene instructions strictly.

Clinical evaluation criteria

The patients' caries status was evaluated in accordance with the ICDAS criteria. The salivary parameters were evaluated by the flow rate, buffer capacity, and the bacterial count of *Streptococcus mutans* (SM) and *Lactobacillus* (LB). Two dentists with at least 10 years of experience identified and classified caries lesions in accordance with the ICDAS-II criteria.[19] The intraexaminer and interexaminer reliability was calculated using the Cohen kappa statistic. The kappa scores were 0.85 and 0.92 for first and second examiners, respectively. The interexaminer score was 0.85, which indicated a high level of agreement.[14,20] For each patient at each appointment, professional tooth cleaning was performed on the occlusal and cervical surfaces by a rotating bristle brush before the clinical examination. At the baseline and at 6 months, each tooth was independently examined using a periodontal probe, mirror, and air syringe under adequate dental chair illumination by two calibrated dental examiners. Clinical examinations were performed and recorded without reference to previous records for each appointment. If the two examiners had any disagreement for any evaluation, a final consensus was reached after discussion at the time of the examination.

Salivary flow rate measurement

Stimulated whole saliva samples from the test and control group patients at baseline and at the end of the study period were collected in relaxed condition between 9 a.m. and 12 p.m. Patients first chewed a piece of paraffin wax (approximately 2 g) for 30 s, and then they did spit it out.[21] Immediately after, saliva was collected into a dry, millimetric container for 5 min by chewing the paraffin wax during this time. The collected saliva volume was measured, and the flow rate was calculated by milliliters per minute. To minimize the effects of the salivary composition, saliva samples were collected from the patients at least 2 h after meals and 1 h after drinking, smoking and tooth brushing, or using mouth rinse.[21]

Salivary buffer capacity measurement

Saliva buffer capacity for each evaluation period was assessed by a hand-held pH meter (LAQUA Twin pH Meter; Horiba Ltd., Kyoto, Japan). To ensure correct measurements, the calibration of the pH meter was checked by a standard buffer for every ten tests. To measure the collected stimulated whole saliva, the saliva samples (0.5 ml each sample) were first placed onto the pH electrode to measure their initial pH value.[22] One milliliter of 0.1 N hydrochloric acid was thereafter added into the saliva samples through the open slide cover of the pH meter. After waiting a few seconds, the pH value was recorded.[22] The buffer capacity was calculated according to the pH changes.

Microbiological evaluation

A standard microbiological analysis kit (CRT Bacteria; Ivoclar-Vivadent, Schaan, Liechtenstein) was used to determine the SM and LB bacterial levels. Saliva samples were dropped onto both sides of SM and LB agar plates in the CRT Bacteria kit by a pipette, and the excess saliva was allowed to drip off. After 48 h incubation at 37°C, the level of viable SM and LB in each sample was measured, and the bacterial counts in saliva were determined by the number of colony-forming units per milliliter (CFU/ml) of saliva using the model chart in the bacteria kit.

Statistical analysis

The obtained ICDAS scores, independent of the surface, were classified as the total number of lesions for each patient divided by the number of teeth, which resulted in a proportionate score, as previously described.[14] Because the obtained results did not show a normal distribution (Kolmogorov–Smirnov test), the data were analyzed between the evaluation periods by using the nonparametric Mann–Whitney U-test, Wilcoxon signed rank test and the McNemar's Chi-square test at the 5% significance level. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software, version 21 (IBM SPSS Statistics, Chicago, IL, USA).

Results

Forty-five patients for the test group with a mean age of 21.7 years (standard deviation [SD]: 4.21 years, range: 17–25 years) and 45 patients for the control group with a mean age of 20.4 years (SD: 3.20 years, range: 17–24-year-old) met the inclusion criteria and were enrolled in the study at baseline. After the initial dental examination, the test group patients started isotretinoin therapy (0.5 mg/kg/day) for 6 months. The control group patients were evaluated by dental examiners based on the evaluation criteria. Table 1 summarizes the characteristics of both patient groups and the significance of the evaluation criteria at baseline. After the 6 months

Table 1: Characteristics and significance of the test and control group patients at baseline

	Baseline		*P*
	Test group	Control group	
Males	14	12	0.82
Females	31	33	0.85
Mean age	21.77±4.21	20.44±3.20	0.511
Overall ICDAS scores per group (%)	25.90±12.94	24.16±9.36	0.637
Salivary flow	1.30±0.67	1.14±0.58	0.575
pH	5.43±0.47	5.28±0.57	0.306

ICDAS=International Caries Detection and Assessment System

evaluation, 12 patients from the test group could not be evaluated because they refused to continue dental examinations and one patient had relocated to another city. The dermatologists withdrew ten patients because of the adverse effects of the medication (e.g., increased serum levels of liver enzymes and lipid changes). Thus, at the end of the study, 22 patients from the test group successfully completed the study for evaluation. For the control group patients, eight patients could not be evaluated because they refused to continue the dental examinations. The dental examiners withdrew 19 patients because eight patients had undergone treatment for their teeth during the study period and 11 patients refused to give a saliva sample for the 6-month evaluation. Thus, 18 patients from the control group completed the full course of the study for evaluation. The flow chart of the study is given in Figure 1. At baseline, no significant differences were observed between the patient groups for each evaluation parameter ($P > 0.05$). When evaluated parameters were compared in detail between the test and control group patients at baseline, the ICDAS scores, which were recorded as a proportionate score per patient, showed no significant difference ($P = 0.637$). In addition, salivary flow (1.30 ± 0.67 ml/min and 1.14 ± 0.58 ml/min, respectively; $P = 0.575$) and buffering capacity (5.43 ± 0.47, 5.28 ± 0.57, respectively, $P = 0.306$) were also insignificant [Table 1]. The Chi-square test also revealed that the mean level of the patients' SM and LB counts was insignificant at baseline in the test and control groups ($P > 0.05$) [Table 2].

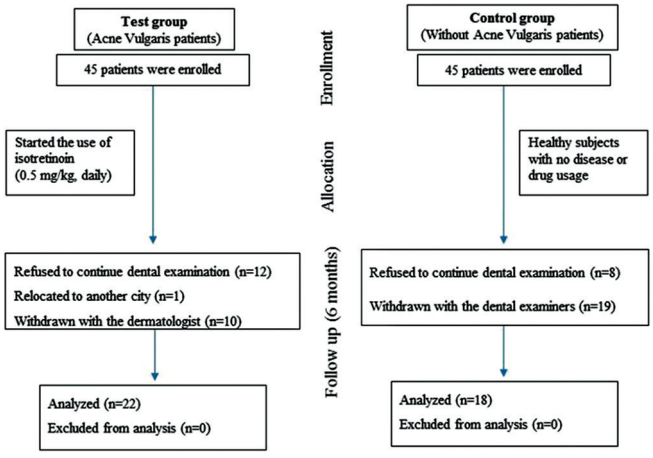

Figure 1: The flow chart of the study

At 6 months, when the proportionated ICDAS scores were compared between the test and control group patients for changes from the baseline values, the test group showed significant differences ($P < 0.05$). However, the control group showed no significant differences ($P > 0.05$) [Table 3]. During the study period, stimulated salivary flow significantly decreased from 1.30 ± 0.67 ml/min to 1.13 ± 0.64 ml/min in the test group ($P < 0.05$). It was relatively increased in the control group from 1.14 ± 0.58 ml/min to 1.19 ± 0.51 ml/min but with no significant differences ($P > 0.05$). At 6 months, when the buffer capacity of both groups was compared for changes from the baseline values, the test group showed significant differences ($P < 0.05$); however, the control group showed no significant changes ($P = 0.655$) [Table 3]. When the change of salivary flow and buffer capacity were compared, statistically significant differences were observed between the test and control groups ($P < 0.05$) [Table 4]. At 6 months, when bacteriological analyses were compared for changes from the baseline values between the test and control group patients, no significant differences were observed with respect to the total number of patients representing more and/or $<10^5$ CFU/ml bacteria in the saliva ($P > 0.05$) [Table 2].

Table 5 summarizes the Spearman's rank correlation results of the salivary parameters and proportionated ICDAS scores at baseline and at 6 months for both groups. The investigated salivary parameters were not correlated significantly with the ICDAS scores tested.

Table 6 summarizes the enter method logistic regression analyses results between the groups. When all of the

Table 3: Significance and differences between end and baseline of the test and control group patients according to the parameters for ICDAS scores, Salivary Flow and pH

	Test group	*P*	Control group	*P*
$ICDAS_{end}$-$ICDAS_{baseline}$	−2.527	0.012	−1.890	0.059
Salivary $Flow_{end}$-Salivary $Flow_{baseline}$	−2.204	0.028	−1.582	0.114
pH_{end}-$pH_{baseline}$	−2.762	0.006	−0.447	0.655

ICDAS=International Caries Detection and Assessment System

Table 2: Comparison and significance of pathogen counts (St. Mutans and Lactobacillus) for the test and control group patients

Groups	$St.\ Mutans_{baseline}$		$St.\ Mutans_{end}$		*P*	$Lactobacillus_{baseline}$		$Lactobacillus_{end}$		*P*
	$<10^5$	$>10^5$	$<10^5$	$>10^5$		$<10^5$	$>10^5$	$<10^5$	$>10^5$	
Test	8	3	4	7	1.000	4	6	5	7	1.000
Control	5	3	1	9	0.625	4	2	1	11	1.000

Table 4: Change of salivary flow, buffer capacity and significance between groups

Parameter	Group	Mean±SD	Median	Percentile 25	Percentile 75	*P*
Change of salivary flow	Test	0.17±0.54	0.15	0.00	0.40	0.002
	Control	−0.04±0.11	0.00	−0.10	0.00	
Change of buffer capacity	Test	0.27±0.37	0.00	0.00	0.50	0.009
	Control	−0.03±0.27	0.00	0.00	0.00	

Mann-Whitney U test, $P<0.05$. SD=Standard deviation

Table 5: Spearman's rank correlation test results and significance between the salivary parameters, ICDAS scores as a proportion with respect to both groups for each evaluation period

Group	Salivary parameters	$ICDAS_{baseline}$	$ICDAS_{end}$
Test	$Salivary\ flow_{baseline}$	r=0.172	r=0.189
		P=0.443	*P*=0.399
	$Buffer_{baseline}$	r=0.074	r=0.158
		P=0.742	*P*=0.481
	$Salivary\ flow_{end}$	r=0.205	r=0.232
		P=0.361	*P*=0.298
	$Buffer_{end}$	r=0.001	r=0.019
		P=0.996	*P*=0.933
Control	$Salivary\ flow_{baseline}$	r=0.041	r=0.059
		P=0.870	*P*=0.817
	$Buffer_{baseline}$	r=0.061	r=0.157
		P=0.789	*P*=0.533
	$Salivary\ flow_{end}$	r=0.046	r=0.136
		P=0.855	*P*=0.591
	$Buffer_{end}$	r=0.130	r=0.247
		P=0.608	*P*=0.324

ICDAS=International Caries Detection and Assessment System

Table 6: The results of the enter method logistic regression analyses

Variable	*B*	SE	Wald	df	*P*	Exp(B)	95% CI for Exp(B)	
							Lower	Upper
Age	−0.064	0.104	0.381	1	0.537	0.938	0.766	1.149
Change of salivary flow	−1.501	1.240	1.465	1	0.226	0.223	0.020	2.533
Change of buffer capacity	−3.059	1.862	2.699	1	0.100	0.047	0.001	1.805
Change of ICDAS scores (%)	0.231	0.225	1.053	1	0.305	1.260	0.810	1.959
Constant	1.689	2.241	0.568	1	0.451	5.412	-	-

ICDAS=International Caries Detection and Assessment System; SE=Standard error

significant variables were entered in the regression analyses, age (odd ratio = 0.938, $P = 0.537$, confidence interval [CI] = 0.766–1.149), change of salivary flow (odd ratio = 0.223, $P = 0.226$, CI = 0.020–2.533), change of buffer capacity (odd ratio = 0.047, $P = 0.100$, CI = 0.001–1.805), and change of ICDAS scores (%) (odd ratio = 1.260, $P = 0.305$, CI = 0.810–1.959) were found to be insignificantly associated with caries occurrence.

Discussion

The findings of this clinical study did not support the first null hypothesis. The caries lesion activity that was evaluated by the ICDAS visual scoring system, salivary flow, and buffer capacity in the test group at 6 months had significantly changed from the baseline values; however, the control group had no significant differences using the same evaluated parameters. No significant microbiological change was observed for the test and control group during the evaluation period.

In the present study, the total percentage score of lesions in the test group significantly changed from the baseline scores. This could be because of the significant decrease in salivary flow and in the buffer capacity of the test group patients led to the development of new incipient caries lesions and the progression of the precavitated

caries lesions. Previous reports indicate that low buffering capacity is interrelated with a low salivary flow,[21] and decreased buffer capacity significantly influences caries progression in adolescents.[21,23]

Salivary parameters such as flow and buffer capacity have a significant role in maintaining oral health such as protecting teeth from caries risk and oral soft tissue from infections.[9,12,24] Studies confirm that decreased salivary flow in patients results in susceptibility to dental caries.[9,13,14,25] Previous studies also confirm that medication can decrease the salivary flow rate.[5-8] The results of the present study are in accordance with the previous studies in that, at the end of the medication period, salivary flow and buffer capacity in the test group were significantly reduced, compared to baseline.[7,8] The change of salivary flow and buffer capacity between test and control groups showed statistically significant differences in the present study. No significant changes were observed for the controls in relation to salivary parameters. Until now, the exact mechanism of action for isotretinoin is not clear; however, the change of salivary parameters may be because systemic isotretinoin therapy reduced the activity and size of sebaceous glands and decreased the quantity of sebum as previously reported.[26] In addition, the use of isotretinoin over the course of the study significantly reduced the salivary gland functions, affected the salivary flow as reported in a recent radionuclide imaging study.[27] Lupi-Pégurier *et al*.[8] have also reported that isotretinoin produced a significant salivary flow reduction from baseline to the end of treatment in their controlled, prospective study.

Stimulated salivary flow is a good evaluation parameter for testing salivary gland function due to its reproducibility.[14,21] When salivary flow is below 0.7 ml/min, it is described as "hyposalivation."[14,28] In the present study, stimulated salivary flow was used to evaluate salivary parameters, and the salivary flow in all patients was higher than the described hyposalivation threshold.

An acidic environment resulting from bacterial carbohydrate metabolism – especially sucrose around the tooth surface – diminishes the pH to the critical threshold (i.e., <5.5) and results in demineralization of the enamel.[14,29] The salivary buffer capacity is important for maintaining the pH values in the oral environment above the critical threshold to buffer the acidic products and increase the saturation of calcium and phosphate ions in the saliva, thereby protecting teeth against demineralization.[22,24,30] At the end of the study period in the present study, the salivary buffer capacity was significantly decreased from the baseline values in the test group, compared to the controls. This may be because using isotretinoin for 6 months significantly affected the buffering capacity of the saliva and led to an imbalance in buffering acidic compounds and consequently demineralization of the teeth.[8]

Microbiological conditions, especially the counts of SM and LB, are associated with a risk of caries in individuals.[8] There was an increase in the number of patients representing more than 10^5 CFU/ml bacteria in the saliva, but there was interestingly no significant difference for either patient group at the baseline and at 6 months. Therefore, we assume that the use isotretinoin in the treated group did not modify the bacterial counts in saliva, as previously reported.[8]

To the best of our knowledge, this is the first study evaluating the association between the salivary flow, buffering capacity, and caries status of the patients who undergo isotretinoin therapy. The results of this study showed no correlations between the salivary parameters and ICDAS scores for the duration of the period. Therefore, we accepted the second null hypothesis of this study.

In the present study, although bivariate analysis showed a significant risk factor in the evaluated ICDAS scores, salivary flow and buffer capacity parameters for the test group compared to baseline values, whereas these factors including individuals' age were found to be insignificant when evaluated directly with the simultaneous impact on the caries occurrence after the enter method logistic regression analyses.

There were limitations to this study. One limitation is the decrease in power for clinical significance resulting from the significant dropout of patients. The insignificant correlation between the salivary parameters and caries activity in the treated patients group may have resulted from this. Therefore, further studies with real power and larger populations require validating the obtained results from this investigation.

Conclusions

Within the limitations of the present study, we concluded that the use of isotretinoin in acne vulgaris patients significantly affected the salivary flow, buffer capacity, and caries lesion activity compared to control patients for whom no significant changes were observed with the same evaluation parameters for the duration of the study. Isotretinoin use has no significant effect on intraoral pathogen microorganism counts. Salivary parameters (i.e., salivary flow and

buffering capacity) showed no correlations with potential caries lesion activity (scored using ICDAS). A limited number of patients were investigated in this study; however, isotretinoin therapy affected the patients' oral health condition. Therefore, dermatologists should be aware of the potential intraoral adverse effects of this medication. Before initiating this drug, they must advise the indicated patients to have frequent dental follow-up visits for oral hygiene instructions, professional oral hygiene regimens, fluoride treatment, and follow dietary instructions to prevent accelerated caries development during isotretinoin therapy.

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Conflicts of interest

There are no conflicts of interest.

References

1. Purdy S, Deberker D. Acne vulgaris. BMJ Clin Evid 2008;2008. pii:1714.
2. Williams HC, Dellavalle RP, Garner S. Acne vulgaris. Lancet 2012;379:361-72.
3. Garner SE, Eady A, Bennett C, Newton JN, Thomas K, Popescu CM. Minocycline for acne vulgaris: Efficacy and safety. Cochrane Database Syst Rev 2012;8:CD002086.
4. Lee YH, Liu G, Thiboutot DM, Leslie DL, Kirby JS. A retrospective analysis of the duration of oral antibiotic therapy for the treatment of acne among adolescents: Investigating practice gaps and potential cost-savings. J Am Acad Dermatol 2014;71:70-6.
5. Scully C. Drug effects on salivary glands: Dry mouth. Oral Dis 2003;9:165-76.
6. Reynolds NJ, Gough M, Clamp JR, Burton JL. Effect of oral isotretinoin therapy on saliva volume and composition. Br J Dermatol 1991;125:189-90.
7. Oikarinen K, Salo T, Kylmäniemi M, Palatsi R, Karhunen T, Oikarinen A. Systemic oral isotretinoin therapy and flow rate, pH, and matrix metalloproteinase-9 activity of stimulated saliva. Acta Odontol Scand 1995;53:369-71.
8. Lupi-Pégurier L, Muller-Bolla M, Fontas E, Ortonne JP. Reduced salivary flow induced by systemic isotretinoin may lead to dental decay. A prospective clinical study. Dermatology 2007;214:221-6.
9. Stookey GK. The effect of saliva on dental caries. J Am Dent Assoc 2008;139 Suppl:11S-7S.
10. Preethi BP, Reshma D, Anand P. Evaluation of flow rate, pH, Buffering capacity, calcium, total proteins and total antioxidant capacity levels of saliva in caries free and caries active children: An *in vivo* study. Indian J Clin Biochem 2010;25:425-8.
11. Rugg-Gunn A. Dental caries: Strategies to control this preventable disease. Acta Med Acad 2013;42:117-30.
12. Humphrey SP, Williamson RT. A review of saliva: Normal composition, flow, and function. J Prosthet Dent 2001;85:162-9.
13. O'Sullivan EA, Curzon ME. Salivary factors affecting dental erosion in children. Caries Res 2000;34:82-7.
14. Varma S, Banerjee A, Bartlett D. An *in vivo* investigation of associations between saliva properties, caries prevalence and potential lesion activity in an adult UK population. J Dent 2008;36:294-9.
15. Broadbent JM, Thomson WM. For debate: Problems with the DMF index pertinent to dental caries data analysis. Community Dent Oral Epidemiol 2005;33:400-9.
16. Pitts N. "ICDAS" – An international system for caries detection and assessment being developed to facilitate caries epidemiology, research and appropriate clinical management. Community Dent Health 2004;21:193-8.
17. Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, *et al.* The International Caries Detection and Assessment System (ICDAS): An integrated system for measuring dental caries. Community Dent Oral Epidemiol 2007;35:170-8.
18. Akarslan ZZ, Sadik B, Sadik E, Erten H. Dietary habits and oral health related behaviors in relation to DMFT indexes of a group of young adult patients attending a dental school. Med Oral Patol Oral Cir Bucal 2008;13:E800-7.
19. International Caries Detection and Assessment System (ICDAS) Coordinating Committee. Criteria Manual: International Caries Detection and Assessment System (ICDAS-II), Bogota, Colombia and Budapest, Hungary; 2009. Available from: http://www.icdas.org. [Last cited on 2010 Sep 20].
20. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33:159-74.
21. Moritsuka M, Kitasako Y, Burrow MF, Ikeda M, Tagami J, Nomura S. Quantitative assessment for stimulated saliva flow rate and buffering capacity in relation to different ages. J Dent 2006;34:716-20.
22. Aiuchi H, Kitasako Y, Fukuda Y, Nakashima S, Burrow MF, Tagami J. Relationship between quantitative assessments of salivary buffering capacity and ion activity product for hydroxyapatite in relation to cariogenic potential. Aust Dent J 2008;53:167-71.
23. Bruno-Ambrosius K, Yucel-Lindberg T, Twetman S. Salivary buffer capacity in relation to menarche and progesterone levels in saliva from adolescent girls: A longitudinal study. Acta Odontol Scand 2004;62:269-72.
24. Sreebny LM. Saliva in health and disease: An appraisal and update. Int Dent J 2000;50:140-61.
25. Atkinson JC, Grisius M, Massey W. Salivary hypofunction and xerostomia: Diagnosis and treatment. Dent Clin North Am 2005;49:309-26.
26. Rodrigues MT, Albuquerque DF, Capelozza AL, Faria FA, Santos CF. Oral side effects of isotretinoin chronic intake. N Y State Dent J 2008;74:36-9.
27. Örsal E, Seven B, Erdem MT, Varoglu E, Farimaz H, Ayan AK. Evaluation of the effect of isotretinoin on salivary gland function by Tc-99m pertechnetate imaging in acne vulgaris patients. Turk J Med Sci 2015;45:674-7.
28. Tenovuo J. Salivary parameters of relevance for assessing caries activity in individuals and populations. Community Dent Oral Epidemiol 1997;25:82-6.
29. Anderson P, Hector MP, Rampersad MA. Critical pH in resting and stimulated whole saliva in groups of children and adults. Int J Paediatr Dent 2001;11:266-73.
30. Erdem V, Yildiz M, Erdem T. The Evaluation of saliva flow rate, pH, Buffer capacity, microbiological content and indice of decayed, missing and filled teeth in Behçet's patients. Balkan Med J 2013;30:211-4.