Journal of Advances in Medicine and Medical Research

32(2): 127-133, 2020; Article no.JAMMR.54666
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Expression of Salivary Resistin in Oral Premalignant Individuals

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Authors' contributions

This work was carried out in collaboration among all authors. Author SSR conceived the idea, did the bench work, data collection and wrote the manuscript. Author AR helped in reviewing and did the proof reading. Author MS helped in sampling and bench work. Author SB helped in reviewing and designing the project. Author HB facilitated in data collection. Author MH overall supervised the project and finalized the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2020/v32i230376
Editor(s):
(1) Dr. Armando Montesinos Flores, National Autonomous University of Mexico, Mexico.
(2) Dr. Thomas I. Nathaniel, University of South Carolina, USA.
Reviewers:
(1) Embolo Enyegue Elisee Libert, University of Douala, Cameroon.
(2) ASV Prasad, Gitam University, India.
(3) Arthur N. Chuemere, University of Port Harcourt, Nigeria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/54666

Original Research Article

Received 22 December 2019
Accepted 28 February 2020
Published 10 March 2020

ABSTRACT

Introduction: Resistin, (Retn) a pro - inflammatory cytokine, accumulates at the site of inflammation. It is found to be elevated in chronic inflammation. The aim of this present study is to evaluate the levels of salivary resistin in healthy individuals and in patients with Oral pre-malignant lesion.

Materials and Methods: This case control study comprises of total 90 patients which includes 45 healthy controls and 45 cases diagnosed with oral pre-malignant lesions. Salivary levels of resistin and clinical parameters were evaluated in all of them. Socio - demographic data (age, gender and residence) was collected from all participants through a questionnaire. In addition, we also recorded the total duration of tobacco usage (in years), daily frequency, and intra-oral examination and oral hygiene practices. This was followed by evaluation of clinical parameters of oral premalignant lesions and investigation of salivary levels of resistin through ELISA.

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Results: The saliva of all the patients showed presence of resistin. On analyzing the samples present study shows no significant difference and variation in the salivary levels of resistin in healthy and OPML patients.
Conclusion: With the results of this study, it can be concluded that there is no significant difference in the salivary levels of resistin in healthy individuals and individuals diagnosed with oral pre-malignant lesion.

Keywords: Resistin; oral pre-malignant lesion; saliva.

1. INTRODUCTION

A biomarker is estimated and evaluated as an indicator of a normal biological process, pharmaceutical response to therapeutic intervention or pathogenic process [1,2]. Any physiological or pathological changes in a particular tissue or cell type is indicated by one or more biomarkers secreted by tumor itself or by other tissues during the development of oral pre-malignant lesion [3].

Cytokines are intercellular signaling proteins [4] which binds with specific cell membrane receptors and influence a number of activities such as normal growth, tissue repair, cellular differentiation, cellular proliferation and angiogenesis [2,5]. They are also involved in the immune response against infection and inflammation and affect cell behavior in diverse manners [2,4,5]. Cytokines play a major role in suppressing or enhancing oncogenesis and can support in the diagnosis of pre-malignancy [6]. They also provide information about presence of disease, epithelial behavior, local inflammatory response, and carcinogenesis [5].

Resistin, (Retn) a pro - inflammatory cytokine [7], also recognized as ADSF (adipocyte-specific secretory factor) or FIZZ3 (found in inflammatory zone-3) [8], accumulates at the site of inflammation [9]. Being a pro – inflammatory adipocytokine it is found to be elevated in chronic inflammation [10]. It is an essential regulator and local initiator of the inflammatory cytokine cascade mediating its pro-inflammatory effects [11]. Following pro-inflammatory stimuli it is secreted by adipocytes [4] inducing the production of peptidoglycan, endotoxin, [11] Interleukin-6,8,12, and tumor necrosis factor in white adipose tissues [4]. Migration of endothelial cells, cellular growth and differentiation are normal processes; but are equally critical for tumorigenesis and angiogenesis and are promoted by resistin (RETN) [4].

Oral cancer may develop de novo but usually as a two stage process; first being a pre- malignant lesion and the second the development of frank carcinoma. If detected while still in the pre-malignant state the survival rate is 80-90% which decreases to 50% if diagnosed at Stage III and stage IV [12,13]. Early detection of malignancies has the ability to reduce mortality and improve therapeutic access. Resistin plays a key role in innate defense mechanisms [11]. It has unlocked new opportunities for disease-related biomarker discovery [14]. The available literature shows the levels of salivary Resistin in a healthy population and in patients habituated to tobacco exposure with oral pre-malignant lesions, have not been studied in our population so far and levels of this biomarkers may possibly reflect the status of inflammation and progression in oral pre-malignant lesions.

2. MATERIALS AND METHODS

2.1 Study Sites

The present study was conducted in the Dental OPD of Abbasi Shaheed Hospital and Ziauddin University Hospital between January 2019 – December 2019. The Samples were processed at Clinical laboratory, Ziauddin University, North Nazimabad, Karachi.

2.2 Study Population and Sample Size

Total 90 patients were included out of which 45 healthy individuals were be taken as controls and 45 cases include patients presenting with potential pre-malignant lesions.

2.3 Selection Criteria

Demographically matched subjects b/w 18 – 65 years were included in this study. Patients with any history of malignancy, metabolic or immunocompromised disorders, local and systemic inflammatory disease, pregnant and nursing mothers and patients who had used any corticosteroid or immunosuppressant, for any reason within a week prior to saliva collection were excluded.

2.4 Sampling Method

Non-probability consecutive sampling was applied.

2.5 Technics Used

Unstimulated whole saliva was collected early morning appointment (08:00 a.m. to 11.00 a.m.) by passive drooling method [2]. They were asked to rinse the mouth with water firstly in order to remove food residue and wait at least 10 minutes after rinsing to avoid sample dilution. They were asked to sit on a dental chair and allow Unstimulated WS to accumulate for 5 continuous minutes in their oral cavity followed by expectoration into a graded plastic funnel tube. 1 ml of saliva was gathered from every patient. The samples were placed in a container filled with crushed ice immediately following collection and immediately shifted to the research laboratory, Ziauddin University.

Saliva samples were centrifuged at 2500 RPM (Gemmy Industrial Corp., USA) for 20 minutes at 4ºC and the supernatant (around 3 ml) was stored at -80ºC in multiple aliquots. For the quantitative determination of salivary resistin concentration in saliva, (glory science co., ltd) human resistin enzyme linked immunosorbent assay (Elisa) kit was used. Manufacturer's instructions were followed for the indirect Elisa technique being performed. A spectrophotometer (plate reader stat, fax-2100, awareness technology, USA) was used to determine the absorbance by reading the plates at 50nm.This was done twice to check for reproducibility of the results. The detection limit of the kit was 20 ng/l – 6000 ng/l.

2.6 Statistical Analysis

Open Epi, version 3, open source calculator—SSPropor Print with 95% confidence level and 80% power of the test were considered for sample size calculation. Non-probability consecutive sampling technique was used. Statistical analysis was carried out by SPSS (Statistical Package for Social Sciences) version 20. Prior to running inferential analysis, the parameters were checked for normality using Shapiro – Wilk's test and Kolmogorov-smirnov test. Difference in the levels of salivary Resistin were assessed in oral pre-malignant lesion by Student Pooled t – test. Odds Ratio with 95% confidence interval was calculated to find association between exposure and outcome. P value < 0.05 was considered statistically significant.

2.7 Study Participants

It is a Case control study in which 90 individuals were included. 45 healthy individuals are taken as controls and 45 cases include patients presenting with potential oral pre-malignant lesions. The present study was conducted in the dental OPD of Abbasi Shaheed Hospital and Ziauddin University Hospital between January 2019 – December 2019. The Samples were processed at Clinical laboratory, Ziauddin University, North Nazimabad, Karachi.

3. RESULTS

This case control study comprises of total 90 patients which includes 45 healthy controls and 45 cases diagnosed with oral pre-malignant lesions. Salivary levels of resistin and clinical parameters were evaluated in all of them. Socio - demographic data (age, gender and residence) was collected from all participants through a questionnaire. In addition, we also recorded the total duration of tobacco usage (in years), daily frequency, and intra-oral examination and oral hygiene practices. This was followed by evaluation of clinical parameters of oral premalignant lesions and investigation of salivary levels of resistin through ELISA. Among the participants, 58 were males and 32 were females. The mean age of cases and controls were 32.08 ± 10.08 years. Out of 90 patients, 26 belong to orangi town, 13 were from liaquatabad town, 9 from north nazimabad and few were from shireen Jinnah colony, jamshed town, gadap town, steel town etc. as shown in Table 1. Out of 45 cases of oral premalignant lesions 39 patients (86.6%) were diagnosed with oral sub mucous fibrosis (OSF), 3 patients (6.6%) of oral leukoplakia (OL), 1 (2.2%) of Lichen Planus (LP), 1 (2.2%) Actinic cheilitis (AC), 1 patients (2.2%) were found to have Oral Erythroplakia (OE). Total 29 lesions were found to be pigmented, 9 red in color and 7 were found to be white in color. Regarding habits, 22 (24.4%) were reported to be frequent betel nuts users, whereas 12 were pan users,8 were mawa users and few were of gutka, gem and combined user of all of them. The mean time period of tobacco exposure was reported to be 12.9 ± 5.099 years. Most commonly these lesion were found on buccal mucosa (38) followed by buccal mucosa along with tongue and lips because of position of placement CSLT .40% lesions were found to be

single whereas 11.1% lesions were found to be multiple. Surface texture of 20 lesions were to be smooth, whereas 13 individual cases were found to be ulcerated and rough. Out of 45 cases, 19 cases had regular margins whereas rest of them had irregular ones (Table 2). Most of the patients diagnosed with OSF reported to the Dental OPD with the presenting complain of limited mouth opening, burning sensation. On intra-oral examination mean limited mouth opening were found to be 16-18 mm with the presence of fibrous bands anteriorly and posteriorly on the buccal mucosa. Table 3 shows frequency of risk factor and duration of its use.

Table 1. Socio demographic data characteristics of cases and controls

Demographic characteristics		**n (%)**
Age group (years)	18 – 28	34 (37.8%)
	29 - 39	33 (36.7%)
	40 - 50	17 (18.9%)
	51 – 60	5 (5.6%)
	61 – 70	1 (1.1%)
Gender	Male	58 (64.4%)
	Female	32 (35.6%)
Area/Residents	Orangi town	26
	Shireen Jinnah colony	15
	Liaquatabad town	13
	North nazimabad	9
	New Karachi	5
	Others	22

Table 2. Clinical characteristics of cases

Diagnosis	Oral submucous fibrosis (OSF)	39 (86.6%)	0.144**
	Leukoplakia	3 (6.6%)	
	Lichen planus	1 (2.2%)	
	Actinic cheilitis	1 (2.2%)	
	Erythroplakia	1 (2.2 %)	
Color	Red	9 (20%)	0.241**
	White	7 (15.5 %)	
	Pigmented	29 (64.4%)	
Size	Single	35 (77.7%)	0.350**
	Multiple	10 (22.2%)	
Surface texture	Smooth	20 (44.4%)	0.267**
	Rough	13 (28.8%)	
	Ulcerated	12 (26.6%)	
Margins	Regular	28 (62.2%)	0.294**
	Irregular	17 (37.8%)	
Location	Lips	1	0.139**
	Buccal mucosa	35	
	Tongue	1	
	Gingiva	2	
	Buccal mucosa & lips	2	
	Buccal mucosa & tongue	3	
	Buccal mucosa & fauces	1	
Consistency	Firm	34 (75.5%)	0.289**
	Soft	11 (24.4%)	
Contour	Raised	14 (31.2%)	0.201**
	Flat	28 (62.2%)	
	Depressed	2 (2.2 %)	
	Sessile	1 (1.1%)	

Table 3. Frequency of risk factor exposed to, duration of its use

Risk factors		Cases N = 45	Controls n= 45	P value
Different forms of smokeless tobacco consumed	Betel nut	22	0	0.081**
	Mawa	8	0	
	Gutka	3	0	
	Pan	4	0	
	others	8	0	
Time since using tobacco	6 months – 1 year	6	occasionally	0.095**
	1-10 years	26		
	10-20 years	8		
	20-30 years	5		

P value < 0.05 is considered significant
***Pearson correlation test was used to find the correlation*

Table 4. Minimum, maximum and mean SD of resistin levels

	Minimum	Maximum	Mean	Standard deviation	P -value*
Cases	0.91	5.64	1.33	0.79	0.9*
Controls	0.92	2.33	1.15	0.27	0.9*

**P value <0.05 is considered significant*
Pooled t-test was applied

Salivary levels of resistin were analyzed through ELISA of 45 patients with premalignant lesions and 45 healthy individuals. We observed that the mean salivary resistin levels were 1.33 ± 0.79 ng/ml among patients with oral premalignant lesion whereas in healthy individuals mean levels were found to be 1.154 ± 0.27 ng/ml (Table 4).

4. DISCUSSION

To the best of our understanding after going through published literature, this is the first study evaluating salivary levels of resistin in oral pre-malignant lesions and healthy individuals. The study consisted of a subset of 90 individuals from Karachi, Pakistan, divided into cases and controls placing 45 patients in each group.

Each of these two groups was first assessed for the demographic data and clinical characteristics along with usage and duration of smokeless/chewable tobacco products. This was followed by evaluation of salivary resistin in healthy individuals and in patients clinically diagnosed with oral pre-malignant lesions.

In this study, Out of 90 cases and controls we have observed male predominance 58 (64.4%) in our study population. In this study we have also recorded the mean daily frequency and duration of habit use (in years). It was observed that individuals who had consumed areca nut/betel quid/mawa/gutka for more than 12 years have a higher tendency to develop oral pre-malignant lesions which is similar to other studies [15].

Out of 45 cases in our study most were diagnosed with oral submucous fibrosis (39) and were in the age range of 18-30 years indicating a younger age at the time of diagnosis. There was a male proponderence in OSF patients. Similar young age groups and male predilection have been reported in previous studies as well [15-17]; Qureshi,dawani and shaikh, 2013 with mean ages also similar to ours. In the light of the above mentioned results it can be suggested that occurrence of OSF is more common in younger age group. Similar observations have been reported by several local and Indian studies [17,18], [15,16] confirming our results.

In this study we have collected 3 saliva samples of patients diagnosed with leukoplakia, 1 with lichen planus, 1 patient with actinic cheilitis and 1 with erythroplakia as well.

In this study patients diagnosed with leukoplakia showed a female predilection with a ratio of 1:2. Prevalence was found to be 3.3% which is similar to that found in other studies [19].

In this study we have also included and collected the saliva samples of patient diagnosed clinically with Oral lichen planus and erythroplakia. Both the lesions were found in male patients. Reticular pattern of lichen planus was found, bilaterally on buccal mucosal lesions. The diagnosed oral lichen planus patients were areca nut habituees. Similar results were reported by a recently conducted study in Jammu and Kashmir, India [20]. The affected individuals diagnosed with

erythoplakia were pan, gutka and mainpuri chewing habituee for the past 10-20 years.

Present study shows no significant difference and variation in the salivary levels of resistin in healthy and OPML patients ($p < 0.9$). No significant change in trends were observed from healthy individuals to individuals having OPMLs. Similar to few other studies our understandings of the causal role and the underlying mechanisms of resistin in progression of cancer and metastasis remains incomplete. The relationship between resistin and tumorigenesis remains inconclusive [21-23]. As a key inflammatory biomarker and mediator of insulin resistance, resistin may indirectly mediate cancer development through its metabolic effects [24].

Our study has some limitations. First, if this study was performed on much larger scale with larger group of people from different walks of life, it will further confirm the possibility of diagnosing potential malignant transformation. Secondly, this study was performed in a single institute and a single measurement of saliva resistin level was assessed which may cause to error.

5. CONCLUSION

Considering the results of this study, it can be concluded that there is no significant difference in the salivary levels of resistin in healthy individuals and individuals diagnosed with oral pre-malignant lesion.

CONSENT

Informed and written consent was obtained from all patients.

ETHICAL APPROVAL

The study approval was sort from the Ziauddin University Ethics Review Committee (ref. code 0370718SSOM).

ACKNOWLEDGEMENTS

The authors are thankful to the board of advanced studies and research (BASR), Ziauddin University for financial assistance of this research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/54666