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STUDY OF SALIVARY ARECOLINE LEVEL IN ARECA-NUT CHEWERS

Dental Science

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ABSTRACT

Introduction: Arecoline, the main areca alkaloid of the betel nut, is reported to have cytotoxic, genotoxic, and mutagenic effects in various cells. Study was undertaken with objective to assess salivary arecoline level in areca nut chewers as compared to that of nonchewers among age group of 25 to 45 years at Jaipur India

Methodology: The patients reported to the Oral Medicine and Radiology Department of Rajasthan Dental College and Hospital, Jaipur, were selected randomly for the study. The sample consisted of 100 patients among age group of 25 to 45 years of which 50 were areca nut chewers & 50 without any kind of chewing habit (areca nut/betel nut etc) formed the control group. Patient history of habits and clinical findings of subjects were recorded on a pretested, predesigned structured Performa. The quantification of salivary arecoline levels was done by using, Agilent 1200 series (HPLC system Germany) by Reversed-phase chromatography.

Results: Out of 100 patients, 71 were males and 29 were females between the age range of 25– 45 years. A Majority of chewers (82%) were males that mean age of chewers group was 34.00 years while that of control group was 32.36 years. Results of this study found significant quantitative alteration in the form of increased arecoline levels in chewers group and no arecoline level found in control group. Arecoline level was significantly more in males as compared to females and showed positive correlation with increase in age.

Conclusion. Males arecanut chewers were at greater risk of oral pathogenicity which increases with age may as indicated by salivary arecoline level.

KEYWORDS

Betel nut, Areca-nut , Arecoline, Carcinogenicity,

Introduction

Areca nut is the seed of the palm tree Areca catechu, nut has always been referred to as supari or "betel nut chewing", which is the fourth most commonly used psychoactive substance, after caffeine, nicotine, and alcohol. There is an estimated 600 million people chewing betel nut worldwide.[1] It is a common habit and is a means of social interaction in Asia, particularly the, South East Asia, South Pacific islands, Papua New Guinea, Bangladesh, Pakistan, and India .[2] Some serious health effects of areca nut chewing are recognized and the International Agency for Research on Cancer has recently classified regular use of areca nut as being carcinogenic to humans.[3] Areca nut contains the closely related alkaloids arecoline, arecaidine, guvacoline and guvacine.[3] Various preparations of areca nut are prepared in India combined with the leaf of the betel vine, piper betle and slaked lime, other fragrance and sweeteners, and marketed as gutkha and pan masala. A survey done by Indian Dental Association (IDA) found that 10% to 14% of school students and 70% of college going students in Mumbai chew gutkha and pan masala.[4] Areca nut use is an independent risk factor for oral squamous cell carcinoma , as well as for the premalignant conditions leukoplakia and oral submucous fibrosis.[5] Arecoline, the main areca alkaloid of the betel nut, is reported to have cytotoxic, genotoxic and mutagenic effects in various cells. Arecoline causes DNA damage through inducing of reactive oxygen species (ROS) and modulation of Matrix Mettalo Proteinase (MMP-2 & MMP-9) in pathogenesis.[6] It shows strong correlation to the incidence of oral submucosal fibrosis, leukoplakia and oral cancer, and has also been found to impose toxic manifestations in immune, hepatic and other defense systems of the recipient.[7] Hence, Therefore assessment salivary arecoline level in arecanut chewers as compared to that of nonchewers will serves as important markers for future oral carcinogenicity.

Objective:

To assess and compare the salivary arecoline levels in areca nut chewers and non – chewers among age group of 25 to 45 years at Jaipur India.

Methodology

The patients reported to the Oral Medicine and Radiology Department of Rajasthan Dental College and Hospital, Jaipur, were selected randomly for the study. The sample consisted of 100 patients among age group of 25 to 45 years which were again divided into Group A & Group B. 50 patients with areca nut chewing habit as per inclusion criteria formed the group A and group B consisting 50 individuals without any history of any kind of chewing habit (areca nut/betel nut etc) formed the control group. Out of 100 patients, 71 were males and 29 were females between the age range of 25– 45 years.

Inclusion & exclusion Criteria

Areca nut chewers consuming at least 4 times 1 pouch or 15 days in a month or 6 months in a year for more than three years and clinically without any lesions. Patients with history of previous treatment for oral squamous cell carcinoma or with any systemic disorder or on any medication affecting the composition and flow of saliva were excluded from study .

After taking signed informed consent from each patient, history of habits and clinical findings of subjects were recorded on a pretested, predesigned structured Performa. The subjects of both the groups were asked to rinse his/her mouth with deionized water, and then unstimulated whole saliva was collected by spitting into15 ml polyethylene test tubes. Saliva samples were centrifuged at 402x g (3000rpm) and decanted into fresh tubes for snap freezing in liquid nitrogen and storage at -20ºC. The quantification of salivary arecoline levels was done by using, Agilent 1200 series (HPLC system Germany) by Reversed-phase chromatography. Data obtained were

analyzed by using appropriate statistical tests using computer software MedCal 14.0.0. Value P<0.05 was taken as significant.

Results

1. Distribution of study participants according to age and sex:

Table 1: Distribution of study & control groups according to age

Age Group	Chewers (A)		Control (B)		Total	
	No.	%	No.	%	No.	%
25-30	17	34.00	24	48.00	41	41.00
31-35	14	28.00	11	22.00	25	25.00
36-40	10	20.00	7	14.00	17	17.00
41-45	9	18.00	8	16.00	17	17.00
Total	50	100.00	50	100.00	100	100.00
Mean age & SD	34 00, SD 5.95		32.36, SD 5.97		P value = 0.172	

Maximum participants (Table1) belong to age group 25-30 years (41%). Followed by age group 31-35 years (25%). And third highest was in age group 36-40 years & 41-45 years both accounted for (17%). On statistical analysis there was no significance difference found between group A & B. (p = 0.172), both groups were comparable.

Table 2: Distribution of study and control groups according to sex

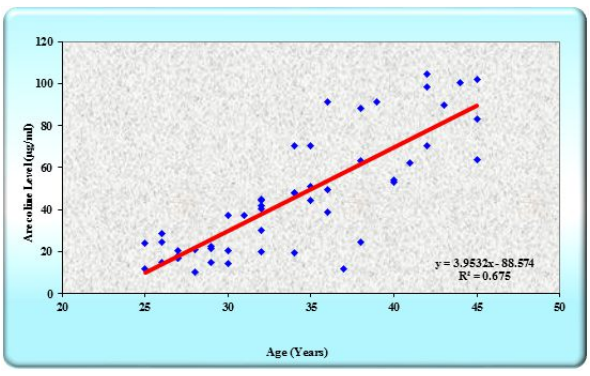
Sex	Chewers (A)		Control (B)		Total	
	No.	%	No.	%	No.	%
Male	41	82.00	30	60.00	71	71.00
Female	9	18.00	20	40.00	29	29.00
Total	50	100.00	50	100.00	100	100.00

Maximum participants (Table 2) were males counted for 71% and females were 29%. mean age of male in chewers group was 34.59 years while that of female was 31.33 years. On application of unpaired T- test this difference found to be statistically non- significant (p= 0.139).

Table 3: Comparison of Arecoline Level according to sex among chewers

Sex	N	Mean arecoline level	Std. Deviation	'p' Value*
Male	41	50.06	28.98	0.024
Female	9	26.56	17.40	

The maximum arecoline level found among chewers group in male was 104.75µg/ml. and in female was 62.39µg/ml. The mean arecoline level in males found to be 50.06µg/ml and in females were 26.56µg/ml. This shows that the arecoline levels in males were higher than females. Mean arecoline levels of both the gender were compared statistically using unpaired T-test and was significant statistically (p value= 0.024).



Graph 1: Correlation Between Age & Arecoline Level in Chewers Group

	Arecoline Level (µg/ml)	'p' Value
Pearson Correlation	0.822	0.0001

The above graph shows that there was good positive correlation ®= 0.822) between age of patient in chewers group and arecoline level and this was found to be statistically significant (p= 0.000).

Discussion

There were many studies done to investigate whether arecanut extract cause carcinogenesis and toxic effect of arecoline as arecoline is a main alkaloid of arecanut. The present study was done to estimate the salivary arecoline level in arecanut chewers & non chewers.

Results of this study shows significant quantitative alteration in the form of increased arecoline levels in chewers group and no arecoline level found in control group. which is similar to the study conducted by Nair et al in 1985[8] In the present study on comparing mean arecoline level with gender among chewers group alone, a significant difference in the mean arecoline levels between male and female gender was noted. The mean arecoline levels were higher in males than females but in this study findings are in contrast to the result to the similar study given by Cox S et al in 2010[7] in which no effect of gender was seen on arecoline levels. But according to the survey study by Kawatra Abhishek et al in 2012[9] the high prevalence rate of arecanut chewers with regards to duration and frequency of chewing were found more in males than females and arecoline level also depends upon frequency of chewing as given by Cox S et al in 2010[7] and in the present study in chewers group the males were 82% and females were 18%. this may be the reason why the arecoline level in male and female genders can give the different variables in different studies.

The present study also shows that on correlating age and arecoline level, the high age group chewers have high arecoline levels. As according to our inclusive criteria the duration of consuming arecanut should be more than 3 years and the chewers should be habitual if the participant is of higher age group and is habitual chewers the frequency of consuming arecanut increases which can results in higher arecoline level which was also proved in similar study given by Cox S et al in 2010[9]

Lee PH. et al in 2006[10] studied that Salivary concentration of arecoline during BQ chewing has been detected to be up to about 140 µg/ml and prolonged exposure to arecoline suppressed the proliferation of human KB epithelial cells. This study suggests that habitual chewers have higher arecoline levels. Many studies[11] have been published on cytotoxic and genotoxic effects of arecanut as it alone can be the risk factor for oral cancer.

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