Efficacy of Crystallization Test in Smoker Patients

[1]Dr.Priyanka B., Lasune, Senior Lecturer,Department of Oral Medicine and Radiology, MIDSR Dental College and Research Center, Latur, Maharashtra.

[2]Dr. Laboni Ghorai, Reader, Department of Oral Medicine and Radiology, Kusum Devi Sundar Lal Dugar Jain Dental College and Hospital, Kolkata.

[3]Dr.Ankita Gupta, Senior Lecturer, Department of Oral Medicine and Radiology, Sree Bankey Bihari Dental College & Research Centre, Ghaziabad, India.

Corresponding Author: Dr. Priyanka B., Department of Oral Medicine and Radiology, MIDSR Dental College and Research Center, Latur, Maharashtra.

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Abstract

Introduction: Crystallization' is a word derived from Crystallography. Crystallography is a special branch of study in science that deals with specific characteristics of crystals of organic and inorganic crystalline substances. This test has been shown a significant role in various head and neck malignancies in mass screening programs. So, the present study is carried out to determine the efficacy of the crystallization test for screening oral malignancy in smokers.

Methods: The study consisted of 100 subjects, 50 healthy individuals and 50 smokers aged between 17-65 years. From each of them, a blood sample was collected by venipuncture under aseptic conditions. The crystallization test was then carried out by mixing the blood sample with cupric chloride solution and incubating the same following all the required protocols. The crystallization patterns obtained was then analyzed.

Result: The crystallization test showed various patterns of crystal formation in different study groups. In the healthy control group, it displayed a typical needle-like arrangement of crystals focusing towards the center and cupric chloride solution alone without admixed blood sample showed up a very haphazard crystal arrangement with complete lack of coordination. Whereas, in smokers, the pattern was " Transverse bar" shaped and Hollow Glan" shaped.

Conclusion: Crystallization test has a great potential in determining risks related to tobacco through different patterns of crystallization.

Keywords: Crystallization pattern, transverse bar, malignancy, screening test, cupric chloride solution.

Introduction

Oral cancer is the eighth most common cause of cancer-related mortality in the world. It is a very heart-mourning situation when we see the distressed faces of the relatives, family members and of course of the patient suffering from cancer. This creates a buzzing alarm to be more aware of the seriousness of this condition as it is preventable if diagnosed at a very early stage and thereby can increase the life expectancy of a patient.

According to World Health Organization [WHO], Cancer is defined as the uncontrolled growth of tissues that is irreversible and persists even after the stimulus has been removed. Smoked and smokeless forms of tobacco have been the known and definite cause of oral cancer. [1,2] Nowadays, the risk of oral cancer increases in males as compared to females due to smoking of tobacco. [3] Tobacco smoking-related diseases can be preventable and this could further decrease the morbidity associated with it. [4] It has been stated in the literature, that each year, approximately 6 million people are buried due to tobacco use and it is estimated that by 2030, the number will rise to 8 million deaths annually. [5, 6] This is because most cases remain undiagnosed until they become visible as a lesion in the oral cavity. [7, 8]

Oral cancer is highly amenable to its prevention. The need of the hour is to study in detail about risk factors in India for the effective prevention and to evoke an evolution in pertinent interventions on preventive measures for oral cancer. Thus, the present study was an attempt to assess signs of malignancy in smokers at an early stage.

Pfeiffer was the first pioneer to introduce crystallization test in search of a simple, economical, minimally invasive technique for cancer detection. This test has proven its competency in assessing and identifying malignancy with uncertain biological significance in oral cancer. [9, 10] This technique attempted in the detection of oral malignancy by observing the different types of crystallization patterns in the cupric chloride solution admixed with the blood of diseased patients. [11] Gulati *et al*. and Kuczkowski et al. were fascinated towards this technique of crystallization in their study because of the weight of its advantages. [10] Gruner et al also tried in identifying different types of crystallization patterns found in both normal and diseased individuals. He identified the role of colloidal protein present in the diluted form of blood which provides different crystallization patterns. [10,12]

Considering this technique's ease of use and advantages, this study was conducted to validate the crystallization test as a screening tool for oral malignancy at an early stage.

Materials and Methods

The study was conducted with a total of 100 subjects, of which 50 were smokers and 50 were healthy patients with no habit of tobacco use. Ethical committee clearance was obtained before the study. The detailed case history was recorded and informed consent was obtained from all patients.

The blood sample was collected from the patients under aseptic conditions. Eight drops of intravenous blood were added on special filter paper (Whatman No.1) on an area approximately 1 cm in diameter and the filter paper was left to dry. The part of the filter paper with dried drops of blood was cut out and dipped in 8 ml of distilled water in a test tube. Four drops of this diluted blood solution were then added to 10 ml of 20% freshly prepared filtered cupric chloride solution in a calibrated test tube and mixed gently. The mixture was poured gently in a flat bottom Petri dish (10 cm diameter) known as ' Assay Petri dish' and placed carefully

inside the crystallization chamber and incubated at 32^0C temperature and 35% to 55% humidity for almost 1 day.

Inclusion criteria

- Male subjects in the age group of 17-65 years
- The individuals who smoke cigarette, bidi and hookah

Exclusion criteria

- The patients who were suffering from systemic diseases
- The individuals who did not give consent

The sample size estimation was effectively done using the formula, $n = \underline{2xZα/2x\ S^2}$

D^2

Statistical Analysis

The documented data were subjected to statistical analysis using SPSS version 21. Chi-square test was used to compare crystallization patterns in healthy patients and smokers. The level of significance was considered at. $P<0.05$.

Results

Figure 1: Crystallization pattern with cupric chloride solution.
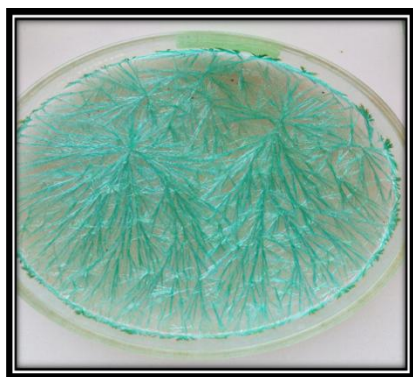
The crystallization pattern of the cupric chloride solution shows a thick crystal with needles arranged randomly along with side branching. Such a pattern was called a muddle formation by Sabarth and Williams.

Figure 2: The E-centric pattern of cupric chloride solution with healthy human blood.

The crystallization pattern in the healthy patient [control group] was eccentrically arranged needles radiating from the centre towards the periphery, as seen in majority of the samples.

Figure 3: Pattern of Transverse Bar Form [a, b] and Hollow Glans formation [c, d] of cupric chloride crystals when blood from smoker patient is combined with cupric chloride solution.

A   B

C   D
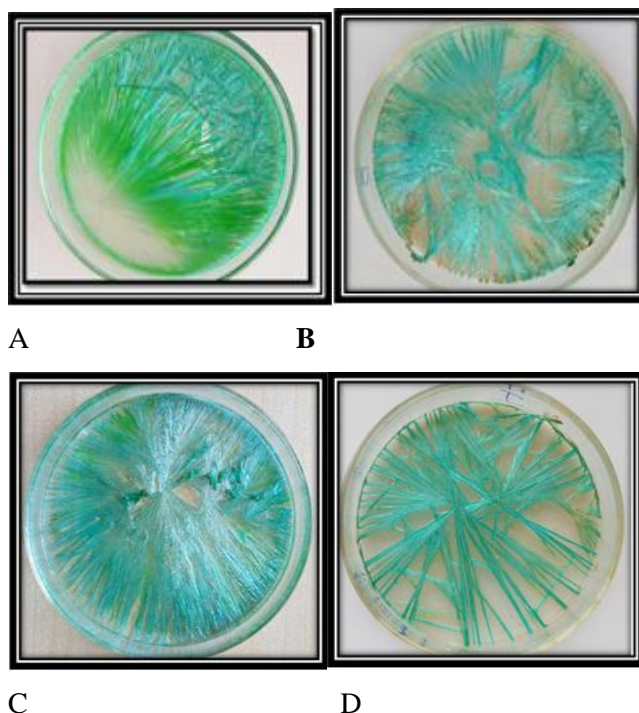
In smoker patients, majority of samples showed the presence of a " Transverse bar" where numerous such transverse bars are present crossing the eccentric pattern and " Hollow Glans" shows multiple basal radiations

and the tangential radiations of crystals on either side of the hollow space till the two meet at the top of the space. Statistically significant result [P<0.001] was obtained when study groups were compared. [Table 1]

Table 1: Study Vs Control Group: (Chi-Square Test)

Group * transverse patterns cross tabulation						
			Transverse Bar And Hollow Glans Patterns		Total	P-Value
			Positive	Negative		
Group	Study Group	% Within Transverse Bar And Hollow Glans Patterns	95.8%	7.7%	50.0%	0.001*
	Control Group	% Within Transverse Bar And Hollow Glans Patterns	4.2%	92.3%	50.0%	
Total		% Within Transverse Bar And Hollow Glans Patterns	100.0%	100.0%	100.0%	

*Statistically significant. P<0.05

*A significant difference was observed in the presence of transverse patterns and Hollow Glansbetween the study and control group. The study group has a higher prevalence of transverse patterns than the control group.

Discussion

Crystallization pattern formation depends on organic and inorganic crystalline substances. [13] Kopaczewski et al. studied crystallization pattern formation and concluded that organic and inorganic salts present in the blood are responsible for the different crystallisation patterns. [12, 9] Earlier, Pfeiffer et al in 1938 experimented crystallization test for the first time and concluded that colloidal proteins existing in the dilute form of human blood behave as an impurity when mixed with cupric chloride solution and get transformed into an orderly pattern with radiating needle-like crystals. These patterns were specific in characteristic in healthy and diseased individuals. [13] Gruner in 1940 concluded that the crystallization pattern varies in healthy and diseased individuals due to the crucial role of colloidal proteins in dilute solution of blood. The particular nature of the abnormal proteins with changes in the position of amino and sulfhydryl groups is responsible for the specific crystallization pattern in the cancer patient. [9,10,12] The

results obtained in our study are similar to those obtained by Pfeiffer et al in his study as both Hollow Gans formations and Transverse bar patterns have been observed here.

In the present study, the crystallization pattern formed with only cupric chloride solution had a bushy appearance (muddle formation) and the same pattern was observed by Selawry [14] and Koopmans, Sabarth and Williams and Sarode et al. and Grishma Rawat in 2018 in their study. The pattern of crystal formation with the blood of healthy patient was eccentric with radiating arrangements from the center to periphery. The same pattern was found in the studies conducted by, Grishma Rawat in 2018, Bali et al. in 2018, Ratna Kumari in 2017 and Sachin Sarode in 2013. [16,17,18,2]

Crystallization patterns formed in some chronic smoker patients showed specific patterns of Transverse bar formation. Similar pattern of Transverse bars was obtained in the study conducted by Bali et al. in 2018, Ratna Kumari in 2017, and Sachin Sarode in 2013 on cancer patients. [2,8] Salawry, Bali S L, and Quadeer reported the " Hollow Glans" Crystallization pattern, which was seen in benign neoplasm irrespective of their origin from basic body tissues like epithelial, connective, muscular and nervous tissues. [14,16] In our study, a similar pattern was found in some of the smoker patients.

The sensitivity and specificity of the crystallization test in screening for oral malignancy were found to be 88.4% and 96% respectively in the study conducted by Ratna Kumari et al in 2018 and in Sarode et al. s study it was found to be 93.55% and 97.66% respectively. Gulati et al. and Kuczkowski et al. in their study, found the sensitivity and specificity to be 88% and 71.5%, respectively, whereas, the study conducted by Shaikh et al. showed 94.7% sensitivity in genital cancer. The

present study shows 98% sensitivity and 95% specificity. This indicates that the test is 97% accurate and can be utilized as a screening method for the early detection of risk of neoplasia.

Conclusion

Crystallization test is a cheaper and quite interesting method for early detection of oral malignancy in high-risk patients. However, more validation is required to confirm the diagnosis. In future, this test can prove to be promising in the mass screening of high-risk individuals who are more prone to develop potentially malignant and malignant oral lesions. Furthermore, this test has great value where the lesion is inaccessible to routine biopsy and other procedures.

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