ORIGINAL RESEARCH

The Potentiality of Crystallization Test in Early Detection of Malignancy in Tobacco Users

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Abstract

Aim: To determine the potentiality of crystallization test in early detection of malignancy in tobacco users as a pilot exploratory observational study.

Methods: 67 subjects were included in the present study. 30 subjects were normal comprising normal group, 30 subjects who were using tobacco for 10 years without any lesions, 5 subjects who were clinically diagnosed as oral premalignant lesion and 2 subjects who were clinically and histopathologically diagnosed as oral squamous cell carcinoma cases were included in the study. All the samples were collected by finger prick in aseptic precautions. Crystallization test was carried out strictly maintaining all necessary conditions.

Results: Cluster pattern was obtained with only cupric chloride alone, radiating pattern was obtained with admixture of normal blood solution and cupric chloride solution and transverse bar pattern was obtained with cupric chloride and blood solution of tobacco users, oral premalignant cases and oral squamous cell carcinoma cases.

Conclusion: As in the result 24 out of 30 cases showed transverse bar pattern, which may suggest a potential association with a risk of transforming into malignancy. Therefore, we can conclude that this test might act as a simple, cost-effective exploratory tool, though further large-scale studies are required to confirm its validity for community-level mass screening.

Keywords: Crystallization Test, Early Detection, Oral Cancer, Tobacco, Pilot Study.

Introduction

Tobacco use is a well-documented risk factor for various forms of cancer, particularly those affecting the oral cavity and respiratory tract. Early detection of malignancy in tobacco users is crucial for improving treatment outcomes and survival rates. Traditional diagnostic methods, such as biopsies and imaging techniques, although effective, often detect cancer at more advanced stages. There is a growing interest in developing non-invasive, rapid, and cost-effective diagnostic tools to identify malignancies at their earliest stages.

The crystallization test, a diagnostic approach with historical roots, has been revisited for its potential in early cancer detection. This test relies on the analysis of crystallization patterns formed when biological fluids, such as blood, are mixed with specific reagents. The hypothesis is that the presence of malignancy alters the biochemical composition of these fluids, leading to distinct crystallization patterns. This method offers several theoretical advantages, including minimal discomfort for patients, ease of administration, and the possibility of frequent monitoring.

This pilot exploratory observational study explores the potential of the crystallization test as an early diagnostic tool for detecting malignancies in tobacco users. By analysing the crystallization patterns of blood samples from both tobacco users and non-users, we aim to determine the test's patterns in relation to early signs of cancer.

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How to cite the article: Naik P., J. Chandrakala, N Sahana, G Suganya, P Akalya, K Anjana. The Potentiality of Crystallization Test in Early Detection of Malignancy in Tobacco Users. Oral MaxillofacPathol J 2026; 17(2); 198-201.

Source of Support: Nil

Conflict of Interest: None

Materials and Method

The study was conducted at the Department of Oral Pathology and Microbiology, Government Dental College and Research Institute, Bangalore, India. The study protocol was approved by the Institutional Ethical Committee of Government Dental College and Research Institute, Bangalore, India (Protocol No. GDCRI/IEC- ACM [2]/5/2017-18). Informed consent was obtained from all normal and test cases group after explaining the procedure.

SAMPLE:

Control group included 30 healthy subjects matched for age and sex having no obvious oral lesions. Test group included 30 cases who were tobacco users from 10 years and the cases were without any lesions. 5 cases who were clinically diagnosed as potentially malignant lesions (3 OSMF and 2 leukoplakia) and 2 cases who were clinically as well as histopathologically diagnosed as oral squamous cell carcinoma were also included in this pilot study. All the cases selected for the study were free from any systemic disease and were in the age range of 30-75 years. Due to the preliminary exploratory nature of this study, a formal statistical sample size calculation was not performed, and a convenience sampling method was utilized.

METHOD: Crystallization test:

Blood sample was collected by pricking ring finger with usual aseptic precautions. One drop (approximately 50 µL) was collected into 1cc of double distilled water at room temperature to give a final dilution of 6 % hemolyzed blood. Four drops of this diluted blood solution were added to 10 ml of 20 % filtered cupric chloride solution in a calibrated test tube and mixed gently. The mixture was poured gently in a flat bottom Petri dish (10cm diameter) known as Assay petridish.

The petridishes were then carefully arranged inside a calibrated hot air oven with strict environmental controls at a constant 35°C for a period of 18- 20 hours for complete crystallization. This test was carried out in all 67 samples (30 normal and 30 test samples, 5 Potentially malignant, 2 OSCC cases). Later the crystallization pattern was studied through hand lens by holding them against the daylight.

STATISTICAL ANALYSIS:

All the data were entered in Microsoft excel and analyzed was using SPSS version 19.0. Chi square test was applied and a $p < 0.05$ was considered as statistically significant.

Results

The present pilot study was conducted to determine the potentiality of crystallization test in early detection of malignancy in tobacco users. The pattern of crystallization with cupric chloride solution alone included thick crystals with needles ar-

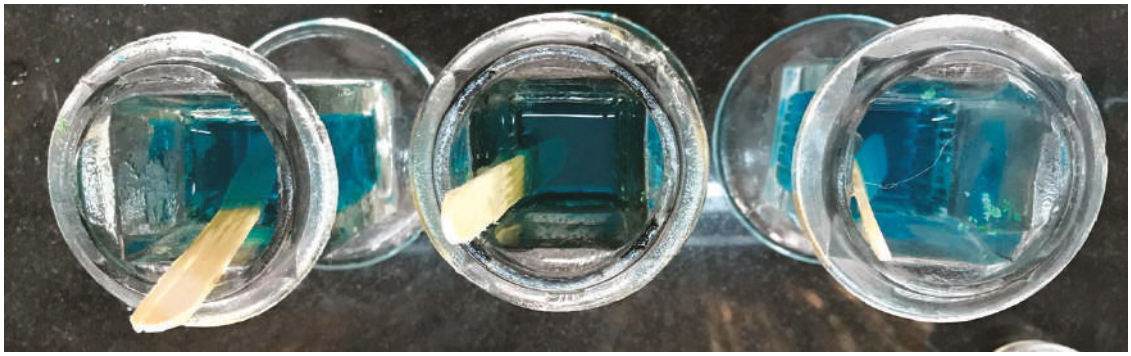

Fig. 1: Hot Air Oven

Fig. 2: Cupric Chloride Solution

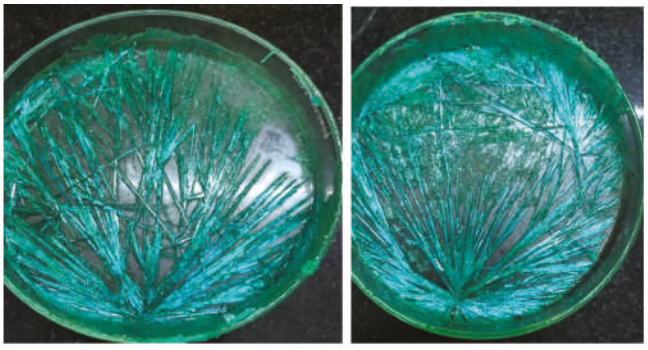

Fig. 3: Crystallization Test in Tobacco Users, & OSCC Group Exhibiting Transverse Pattern

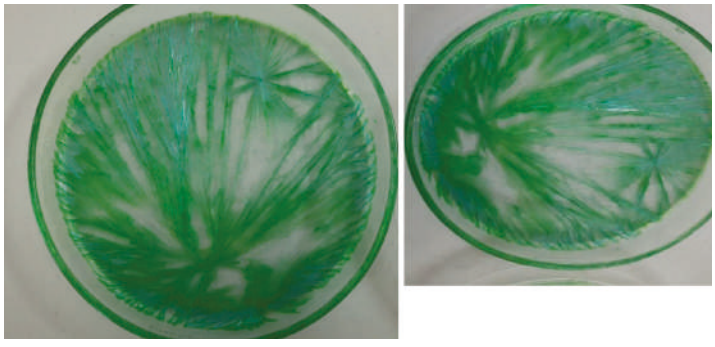

Fig. 4: Crystallization Test In A Normal Individual Exhibiting A Pattern Of Eccentric Centre Of Gravity With Radiating Needles

ranged randomly in haphazard manner. The pattern of crystal formation from blood of control groups presented regularly arranged crystals and showed radiating arrangements from one point called center of gravity. This was located towards one side of the petridish indicating eccentric position.

Among the test cases, potentially malignant cases and OSCC cases, there was presence of transverse bar which consisted of transverse needles with wing like formation on either or both sides respectively. The transverse bar was irregularly distributed.

The above mentioned three patterns observed in the test and control groups are tabulated in table 5. 24 out of 30 test samples showed the transverse bar. The same pattern was observed in potentially malignant and malignant cases. On this pattern, chi square test was applied and the p value came as significant.

Table 1: Pattern of Crystallization Observed:

	Radiating	Transverse Bar	Cluster
CONTROL (n=30)	24(0.8%)	2(0.066%)	4(0.13%)
TEST(n=30)	5(0.16%)	24(0.8%)	1(0.033%)
PMD(n=5)	0	5(100%)	0
OSCC(n=2)	0	2(100%)	0

Table 2: Distribution of Radiating Pattern of Crystallization

	With Radiating Pattern	Without Radiating Pattern	N (%)
CONTROL	24	6	30(80%)
TEST	5	25	30(16.6%)

Table 3: Distribution of Transverse Bar Pattern of Crsytallization

	With Transverse Bar Pattern	without transverse bar pattern	N (%)
CONTROL	2	28	30(0.06%)
TEST	24	6	30(80%)

Table 4: Distribution of Cluster Pattern of Crystallization

	With Cluster Pattern	Without Cluster Pattern	N (%)
CONTROL	4	26	30(0.13%)
TEST	1	29	30(0.03%)

Table 5: Test * Control Cross Tabulation:

Test Group		Transverse Bar Pattern	Radiating Pattern	**Cluster Pattern**	**Total**
Transverse bar pattern	COUNT	2	22	0	24
	% Within test cases	8.3%	91.7%	0.0%	100.0%
	% within control cases	100%	91.7%	0.0%	80.0%
Radiating pattern	Count	0	2	3	5
	% within test cases	0.0%	40.0%	60.0%	100.0%
	% within control cases	0.0%	8.3%	75.0%	16.7%
Cluster pattern	Count	0	0	1	1
	% within test cases	0.0%	0.0%	100.0%	100.0%
	% within control cases	0.0%	0.0%	25.0%	3.3%
	Total count	2	24	4	30
	% within test cases	6.7%	80.0%	13.3%	100.0%
	% within control cases	100.0%	100.0%	100.0%	100.0%

CHI SQUARE TESTS:

	VALUE	Df	Asymptomatic significance (2 sided)
Pearson Chi square	19.708	4	.001
Likelihood ratio	17.164	4	.002
Linear by linear association	13.790	1	.000
N of valid cases	30		

Discussion

Oral cancer accounts for more than 30% of all cancers and poses a significant burden in the Indian subcontinent. Tobacco and betel nut chewing habits are the main contributing factors for the increased incidence of oral potentially malignant disorders as well as oral squamous cell carcinoma. Early identification of high-risk tobacco users before the development of clinically visible oral lesions can reduce the carcinomatous transformation, thereby reducing mortality and morbidity associated with the disease.

Analysis of circulatory body fluids for early cancer diagnosis, prognosis and treatment monitoring are becoming important in clinical practice these days. Biochemical alterations occurring during malignant transformation can be detected in blood as it is an excellent medium reflecting alteration in the body during malignancy.

A specific method for cancer detection was employed by Pfeiffer (1938) in which cupric chloride on admixture with blood of a cancer patient showed specific pattern of crystallization. The principle is 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 of radiating needle-like crystals.

In malignancies there is an increase in the levels of diamines and polyamines in blood which are the intermediates of protein metabolism. These products might be responsible for the specific pattern in crystallization test.

In the present study, the patterns of crystallizations were quite similar to the previous studies. The pattern of crystallization with cupric chloride solution alone included thick crystals with needles that are randomly arranged in a haphazard pattern. The pattern of cupric chloride mixed with blood of healthy subjects consisted of regularly arranged crystals that showed radiating arrangements. Test cases of premalignant and OSCC exhibited transverse bars which consisted of transverse needles with wing like formation on either sides. The reliability was about 80% in the test samples and control group, and 100% among oral potentially malignant and OSCC samples.

Limitations:

This pilot exploratory observational study presents several limitations that must be acknowledged. Foremost is the very small sample size, particularly the subgroups for potentially malignant disorders (n=5) and OSCC cases (n=2). Due to the lack of a formal sample size calculation, the findings are preliminary and cannot be generalized to a broader population. Furthermore, the study design lacked blinding, which may introduce observer bias during the subjective evaluation of crystallization patterns. There is also limited data on reproducibility, an absence of sensitivity and specificity calculations, and a lack of longitudinal follow-up to definitively predict malignant transformation.

Conclusion

The findings of this pilot exploratory observational study underscore the potential of the crystallization test in studying blood sample differences between individuals with and without malignancies and tobacco users. Our analysis demonstrated variations in crystallization patterns, suggesting that this method might identify early biochemical changes. The non-invasive nature of the test positions it as an interesting concept with historical roots.

However, the conclusion that transverse bar patterns definitively indicate malignancy risk must be interpreted with caution due to the extremely limited malignant sample size. Future research should focus on further validating these findings through strictly blinded, larger-scale studies with appropriate longitudinal follow-ups and sensitivity/specificity analyses before considering integration into clinical practice.

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