CRYSTALLIZATION TEST FOR THE DETECTION OF MALIGNANCY OF THE FEMALE GENITAL TRACT

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ABSTRACT
Cancer is an ageold disease which has claimed millions of human lives along with other fatal diseases. Cancer of the cervix is the most prevalent form of cancer in developing countries especially the South-East Asia region.

The present study is undertaken to determine the efficacy of the crystallisation test for the detection of malignancy in cancer cases of the female genital tract and also to determine the efficacy of this test to detect the precancerous conditions (Erosion of cervix etc.) of the female genital tract and to know whether the results of crystallisation test correlate with the histopathological diagnosis of cancer and precancerous cases.

Crystallisation test was carried out in 300 female patients from Gynaecology wards of Government Medical College and Hospital, Aurangabad. Out of 300 cases studied, 211 cases were diagnosed cancer patients. 39 cases were in precancerous stage and 50 were non-cancer cases. The non-cancer cases, including 10 normal cases were taken as a control. Out of 211 diagnosed cancer cases, 92 were histologically proved cancer cases. Positive results were obtained in 94.7% cases of malignancy. Positive results were obtained into 84.6% of precancerous cases.

To all the cancer and non-cancer cases of the female genital tract, Chi-Square test was applied, degree of freedom was calculated and the significance of the test i.e. P value was calculated. P value was found to be far less than 0.001. This indicates that the crystallization test is statistically highly significant test for the detection of malignancy.

Key-words:-- Crystallization, malignancy, precancerous.

INTRODUCTION
The current methods of confirmation of cancerous neoplasms are histopathological, cytological, endoscopic techniques and roentgenic studies. However these studies are valuable only when the growths are obvious. Pfeiffer (1938) advanced a new method for cancer detection by specific crystallization pattern of cupric chloride in admixture with blood of cancer patient.

The present work has been undertaken to detect malignant lesions as well as precancerous lesions eg. (Erosion of cervix etc.) of the female genital tract and to know whether this test is useful where the cancerous lesion is inaccessible to biopsy and other procedures. It is also undertaken to know whether this test is useful for mass screening programmes for cancer and whether it can be utilized as scanning method in cancerous and precancerous conditions.

MATERIAL AND METHODS
When an organic extract is added to a solution of cupric chloride and the resultant mixture allowed to crystallize, the crystals formed assume a pattern, different from that of the crystals which develop from cupric chloride solution alone. The pattern of arrangement of crystals varies with the kind and qualitative characteristic of the organic extract, and is specific for that extract. The present study has shown that a definite arrangement of crystals is produced when blood from normal persons and from patients with malignancy, is added to cupric chloride solution.

Crystallization test was carried out in 300 females from Gynaecology ward of Medical College and Hospital, Aurangabad. Out of 300 cases, 211 were diagnosed cancer cases, 39 were precancerous cases, and 50 were non-cancer cases. The non-cancer cases including 10 normal cases were taken as a control for cancer cases.
Out of 211 diagnosed cancer cases, 92 were histologically proved cancer cases. Crystallization test was carried out in all the cases to know whether it was positive or negative for cancer, whether the localization of crystals in the genital one, was proper or not and whether the results of crystallization test correlate with the histopathological diagnosis of cancer and precancerous cases.

The blood samples were collected from non-cancer patients, histologically proved cancer patients and suspected cancer patients (precancerous cases), by pricking the ring finger with usual aseptic precautions. Eight drops of blood from a cancer patient were allowed to fall on a special filter paper (Whatman No.1) on an area approximately 1 cm in diameter and were left to dry. The parts of the filter paper with dried drops of blood were cut out, and were dissolved in 8 ml of distilled water in a test tube. 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 petridish (10 cm diameter) known as “Assay Petridish”.

Two more petridishes were prepared as control. In one petridish 10 ml of filtered cupric chloride solution was poured. In the other petridish, mixture of 4 drops of blood solution from normal healthy person with 10 ml of filtered cupric chloride solution was poured. The petridishes were carefully arranged inside the crystallization chamber i.e. incubator at 32°C for a period of 19 hours, for complete crystallization under controlled humidity. The test was also carried out in 250 cancer and precancerous cases and 50 non-cancer cases.

**OBSERVATIONS**

Following crystal patterns were observed in the petridishes under study as seen by the dissecting microscope:

1) The crystal pattern with pure cupric chloride solution showed a number of crystals with many centres of gravity. (Figure 2.)

2) The crystal pattern of the mixture of cupric chloride and blood from a normal healthy person showed a single eccentrically placed centre of gravity. (Figure 3.)

3) The crystal pattern of the mixture of cupric chloride with blood from a cancer patient and a precancerous patient showed sharply set off transverse bar formation. (Figure 4 & 5)

The whole crystallization pattern represents the complete human body with definite localization of the various parts of the body, as shown in Figure-1. Accordingly, the crystallization field is divided into four quadrants by drawing vertical and horizontal axes intersecting at centre of gravity which is localized eccentrically in the field towards one side. This is known as the localization map, which outlines the various parts of the body.

In figure-1, only the four quadrants and the genital zone or zone of sexual organs and peripheral or border zone are shown.

![FIGURE-1](image)


The intersection of the A-A and M-M axes = centre of gravity. Number 1, 2, 3, 4 show four quadrants of the crystallization field.

The hatched portion = peripheral or border zone.

S = Genital zone or zone of sexual organs.
**STATISTICAL ANALYSIS**

To all the cancer and non-cancer cases of the female genital tract, Chi-square test was applied, degree of freedom was calculated and the significance of the test i.e. P value was calculated. The P value was found to be far less than 0.001. This indicates that the crystallization test is statistically highly significant test for the detection of malignancy.

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**Table 4** shows that the results of crystallization test correlate very well with the histopathological diagnosis. This indicates that the crystallization test has detected all the proved cancer cases except one which is a false positive result.

**Table-5** shows that the results of crystallization test correlate very less with the histopathological diagnosis. This does not indicate that the crystallization test has failed to detect the precancerous cases but it probably indicates that these cases may develop malignancy at any time in future. Such precancerous cases could not be followed up further because in these cases, operation of total hysterectomy was done.
Table 6 shows that the percentage of proper localization in the cancer and precancerous cases of the female genital tract is 96.4% i.e. all the transverse crystals are localized to the genital zone.

Table 7 shows that the percentage of proper localization in the proved cancer cases of the female genital tract is 98.91% i.e. all the transverse crystals are localized to the genital zone.

Table 8 shows that the percentage of proper localization of crystals in non-cancer cases is 96% i.e. all the crystals are localized to the genital zone.

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From table 6, 7 and 8 it is seen that, out of 250 cancer and precancerous cases of the female genital tract, 241 cases showed proper localization and only 9 cases showed improper localization. Out of 92 proved cancer cases, 91 cases showed proper localization and only 1 case showed improper localization. Out of 50 non-cancer cases, 48 cases showed proper localization and 2 cases showed improper localization. The failure may be attributed to lack of ideal experimental conditions or some fault in technique.
and others have subsequently led to establish a simple test for early detection of cancer, because the positive results were demonstrated in case of susceptible strains of animals even though they were clinically free from any cancerous growth. The patterns which crystals assume after the addition of blood to a solution of cupric chloride under specified conditions have been shown by E. Pfeiffer (1938) to be distinctive between health and disease. In experimental animals, the pattern produced by adding blood to cupric chloride is rendered abnormal and is distinctive and characteristic for each disease. (Gruner, 1940.)

Kopaczewski (1933) has attributed the crystallization pattern to different rates and different amplitudes of molecular movement during the process of evaporation and trikinetic forces operating through a variety of materials. The reaction is mainly physical and no new chemical substance is formed.

The illustrative experiments of Sabarth and Williams (1975), highlight the fact that the dominant role of pattern formation force of some constituents of blood solution, is responsible for the typical crystallization pattern when blood from normal healthy individuals is added to cupric chloride solution. The results of different workers, Pfeiffer and Miley (1939); Morris D.L. and Morris C.T. (1939); Gruner (1940); Selawry (1940); Teichmann (1965); Koopmans (1969); Sabarth and Williams (1975) under different laboratory conditions, have the common feature of similar pattern formation in normal healthy individuals. The same pattern in normal healthy individuals is also observed in the present study.

The crystallization pattern is modified or completely changed when the salts crystallize from solution containing impurities as reported by many workers. [Du Nouy (1926, 1928); Michaud (1936)]

The addition of blood to cupric chloride solution essentially means addition of an impurity. It is very difficult to pinpoint the actual substance acting as an impurity in case of blood as even one drop of blood contains number of vitally important substances like

DISCUSSION

The principle underlying Pfeiffer’s crystallization test is that the colloidal proteins present in an extremely dilute solution of human blood act as an impurity when mixed with cupric chloride solution and are amazingly transformed into well co-ordinated pattern as long radiating needle-like crystals. After a series of experiments he noted that the crystallization pattern of cupric chloride solution differs substantially when the blood of a diseased individual is added to it. Thus he observed star formation in inflammation, a hollow glass in benign condition and transverse formation in cancer. The classical experiments of Gruner (1940)
various ions, hormones, enzymes, proteins and cellular components. Trümpps and Rascher (1936) pinpointed proteinous colloid as the root cause of modified crystallization pattern in biological systems in human beings.

Koepf and Selawry (1962) reported the extreme sensitivity of cupric chloride to proteins. They were of the view that the proteins in colloidal status probably play an important role in the pattern formation mechanism. In cancer, specific pattern of cupric chloride crystals was observed (Pfeiffer 1935, 1939; Gruner 1940; Selawry 1957, 1968; Koef and Selawry 1962; Koopmans 1969). Similar pattern was also observed in the present study.

Bachrach and Plesser (1985) observed elevated levels of naturally occurring polyamines and diamines in the body fluids including blood, in cancer. This information further stresses the positive role of presence of elevated protein levels for detection of cancer. Thus it was confirmed that the protein or the degradation product of protein, is responsible for particular cancer specific pattern, the pattern in inflammation, in allergic or benign conditions and in normal conditions.

Gruner (1940) pointed out that the crystallization test aims at detecting the precursor metabolic changes. Russell (1978) reported high concentrations of polyamines and diamines in cancer cells which are intermediate products of protein metabolism. The excretion of these amines in urine is also found to be elevated in cancer patients. Russell (1971) and Bachrach and Plesser (1985) suggested that these amines could serve as markers of malignancy and probably these are responsible for the positive crystallization test.

Pfeiffer (1938) has demonstrated positive results in 75% of cases, while positive results have been demonstrated in 91.1% cases by Gruner (1940). Quadeer (1980) has demonstrated positive results in 94.15% cases. In the present work, out of 211 diagnosed cancer cases, 200 gave positive results (94.78%) and out of 39 precancerous cases, 33 gave positive results (84.61%). Out of 50 non-cancer cases, only 5 cases showed positive pattern for cancer.

It has been observed in the present study that the percentage of positivity of cancer and precancerous cases in 93.2% which is termed as sensitivity of the test. The percentage of negativity of transverse forms in non-cancer cases is 90% which is termed as specificity of the test.

In the present study, 39 precancerous cases were studied for cancer pattern. Out of 39 precancerous cases, 33 cases showed the typical cancer pattern, while 6 cases failed to give it and the percentage of positivity was found to be 84.61%.

Selawry and his co-workers (1957) studied 52 precancerous cases. Out of 52 precancerous cases, 43 cases showed the typical cancer pattern while 9 cases failed to give it and the percentage of positivity was found to be 82.69%.

Localization of transverse crystals in specific zones of the localization map were also observed in the present study.

Quadeer (1980) studied the localization of crystals in specific zones of the field in cancer cases of various systems and the percentage of proper localization was found to be 90.76%. In the present study, 250 cancer and precancerous cases of the female genital tract were observed for the proper localization of crystals in the genital zone of the localization map, and the percentage of proper localization was found to be 96.4%. The percentage of proper localization of crystals in specific zone in non-cancer cases was found to be 96%.

The positive cases of cancer by the crystallization test were correlated with the histopathological diagnosis of proved cancer cases. Out of 92 proved cancer cases, 91 cases showed positive histopathological diagnosis and only 1 case showed negative result. This shows that the results of crystallization test correlate very well with the histopathological diagnosis. This indicates that the crystallization test has detected all the proved cancer cases except 1 which is a false positive result. It was also observed that the results of crystallization test correlate very less with the histopathological diagnosis of precancerous cases. This does not indicate that the crystallization test has failed to detect the precancerous cases.
but it probably indicates that these cases may develop malignancy at any time in future. Such precancerous cases could not be followed up further, because in these cases, operation of total hysterectomy was done.

**CONCLUSIONS**

At the end of the experiments of crystallization test, the following conclusions were drawn :-

a) Crystallization test can be used for detecting all cancer cases of the female genital tract.

b) Crystallization test is helpful for early detection of cancer i.e. detection of precancerous cases and for detection of cancer in apparently normal individuals with no clinical manifestation of cancer.

c) The great value of this test is in detecting the presence of malignancy of the female genital tract, where the lesion is inaccessible to biopsy and other procedures.

d) Crystallization test helps in providing clue for prognosis.

e) Crystallization test is also helpful in detecting individuals who have genetic predisposition for cancer, long before the appearance of cancerous changes.

**BIBLIOGRAPHY**


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