E-ISSN:2454-9894 P-ISSN: 2454-9886
International Archives of
BioMedical and Clinical Research
(An Official Publication of "Ibn Sina Academy of Medieval Medicine & Sciences")
WWW.IABCR.ORG
Section – Anatomy
Original Article

Crystallization Test for Early Detection of Malignancy

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ABSTRACT

Background: *Cancer can involve any tissue of the body. In most of the cases, patients present themselves to a medical facility when the disease has reached an advanced stage and is not amenable to treatment. So, the line of action in order to cure cancer should be its early detection and prompt treatment of precancerous lesions. This is one of the cornerstones of cancer prevention.* ***Aim:*** *Aim of the study was to determine the efficiency of crystallisation test for early detection of malignancy.* ***Methods:*** *110 subjects were included in the present study. 50 subjects were normal comprising the control group and 60 subjects were diagnosed with dysplastic changes. All samples are collected by finger prick with aseptic precautions. Crystallization tests were carried out strictly maintaining all necessary conditions. The results obtained were than analysed and studied.* ***Results:*** *Crystallization pattern with only cupric chloride solution alone is very haphazard and completely lacking of co-ordination. The pattern of admixture of normal blood solution and cupric chloride is typical and shows co-coordinated arrangement of crystals. Blood crystallization pattern in control group shows very specific arrangement of needle like crystals of cupric chloride. Hollow Glans formation characterizes the benign condition while hollow glans along with gap star formation characterizes the precancerous conditions. Present study of malignant pattern proves that transverse bar or transverse formation is confirmatory finding in advanced cases.* ***Conclusions:*** *Inference of the test shows that control group had shown 100% positivity while all cases of malignant as well as premalignant conditions was shown 99% positivity.*

Key words: *Crystallisation, Cupric Chloride, Malignancy, Benign, Blood, Cancer.*

INTRODUCTION

Cancer is one of the built-in senescent mechanisms an eventual stage in the life cycle of normal diploid dividing cells in a metazoic organisms. It is a time governed process, manifest ongoing of the organism and terminates the life of the organism on its own or with the help of other senescent forces.[1] It may not occur at all or may remain silent despite it's definite presence in the organism.[2]

According to WHO, cancer afflicts all communities worldwide. It is an uncontrolled growth of tissues that is irreversible and persists even after the stimulus has been removed.[3] Cancer can occur at any site or tissue of the body and may involve any type of cells. Unfortunately, in most of the cases, the patients present themselves to a medical facility when the disease is far advanced and is not amenable to treatment.[4]

With vast experience over the years, clinicians have realized that the early detection of cancer is extremely necessary to prolong the life span of cancer patient.[5] Efforts were made around the world to detect the cancer cases at an early stage through scientific advances as well as through social awareness campaign about the disease. [6]

In search of an ideal test for early detection of cancer, it is stated that the test for early detection of malignancy ought to be simple, quick and inexpensive. On screening the literature available in this regard, crystallization test was found to be the nearest option.[7-10]

The present study was undertaken to determine the efficacy of crystallization test for early detection of cancer. The procedure is likely to prove an efficient tool for the early detection of cases which may be susceptible to malignancy in future, and to differentiate between benign and

Access this article online	
Website: www.iabcr.org	Quick Response code
DOI: 10.21276/iabcr.2017.3.2.10	

Received:21.03.17| **Revised**:12.04.17| **Accepted**:15.04.17

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malignant conditions. It can also be used for mass screening of high risk groups.

METHODS

A prospective study was designed to inspect the cases of neoplasia, irrespective of their age, sex and site of lesion which constituted the study group. Control group included subjects who were physiologically fit and belonged to the same age group as the study group. Total number of subjects examined during the study were 110 (n=110), out of which 60 subjects constituted the study group and the other 50 subjects formed the control group. Sixty cases of study group included 2 precancerous lesions, 11 benign lesions and 47 precancerous conditions.

Eight drops of blood obtained by a finger prick were allowed to dry on Whatman filter paper No. 1. Subsequently the dried drops of blood were dissolved in 8 cc of distilled water. 1 cc of such diluted blood solution was added to 20% of cupric chloride solution. This mixture was poured carefully in the Petri dish such that the solution covered the bottom of Petri dish immediately. Five such Petri dishes were prepared for each subject. Petri dishes were carefully arranged in BOD incubator. Crystal formation was allowed to take place up to 24-30 hours at 32.7°c, without any disturbances. After crystallization was complete the dishes were removed and examined. The ideal dishes from each patient were critically studied in the day light, photographed directly and observations were made.

Details of patients were recorded on structural proforma after crystallization. Petri dishes were studied carefully and observations were noted down. Statistical analysis was done by using Chi square test and Fisher's Exact Test.

RESULTS

The blood crystallization patterns are different in health and disease as acknowledged by various workers in the field. So far the most accepted view point is the pivotal role of colloidal proteins in dilute solution of blood. The specific pattern, created by admixture of blood with cupric chloride showed that cupric chloride played an important role in biological activities of the body. Copper is present in various coenzymes of human body and chloride ions are important from osmoregulation.

Crystallization pattern of cupric chloride solution alone revealed varied patterns showing somewhat whorl formation (Fig 1). It showed complicated network and irregular scattered radiations of crystals producing network throughout. Basic structure was thick linear crystals arranged singly or in cluster.

Pattern of crystal formation from blood of control group presented striking contrast of crystal patterns as compared to the cupric chloride solution only. The crystals were regularly arranged and showed radiating arrangements from one point called as center of gravity. This was located not exactly at center but towards one side of the field indicating eccentric position (Fig 2). Due to eccentric position of the centre of gravity, complete field could be divided into two zones. The crystals were arranged in radiating fashion. In major part crystals were long and this part called as zone of long radiations, the remaining part of petri dish called as zone of short radiations.

Crystallisation pattern of benign conditions showed typical hollow glans pattern. It consisted of basal radiations and the tangential radiations of crystals on either side of the hollow space till the two met at the top of the space (Fig 3).

Crystallization pattern in precancerous conditions showed complete absence of node formation, centre of gravity and fanning of crystals were completely absent whereas crystals were arranged in such a way that it had an appearance of hollow glans formation or gap star formation and side branching of crystals or combination of both (Fig 4).

Crystallization pattern in malignancy showed characteristic pattern of transverse bar formation in relation to the radiating crystals of basic pattern. Numerous such transverse bars were seen in case of chronic myeloid leukemia and malignant melanoma. The transverse bars were irregularly distributed (Fig 5).

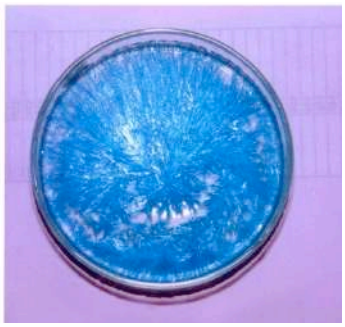

Fig 1: Pattern of only Cupric Chloride crystals-Close view

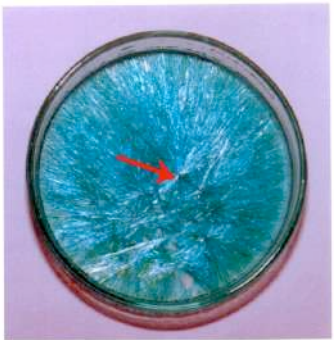

Fig 2: Pattern of cupric chloride in combination of human blood

Fig 3: Crystallization of blood from Benign Condition

Fig 4: Crystallization pattern of blood from Leucoplakia

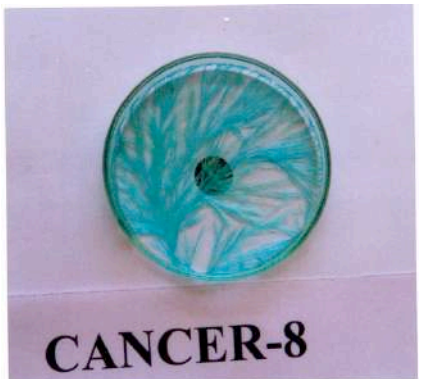

Fig 5: Crystallization Pattern of blood from CA Pineal Gland

DISCUSSION

The process of crystallization pattern of blood attracted the research workers across as early as 1648.9 Glauber studied the crystallization process in mid 17th century and concluded that it is controlled by some "occult" forces. The crystallization test is based purely on physical phenomenon; hence it was very necessary to have controlled physical conditions, which were provided by the B.O.D. incubator.[11]

Crystallography is a special branch of study in science which deals with specific characters of crystals of organic and inorganic crystalline substances.[12] The reaction is physical in nature. Various organic and inorganic substances were added to colloidal solutions, and the resulting patterns were studied.[11] Workers such as Alexander, Du Nouy, Marriage, Michand and Merten stated that the test had diverse application in routine life to identify the adulterants in commercial (Jams and Jollies) or in biological tests for serum etc.[13,14]

In the present study, it was seen that crystallization pattern from cupric chloride solution was found to be showing irregularly scattered radiations with overlapping of crystals along with bushy appearance. The pattern observed by Pfeiffer consisted of baseline of crystals (long or short) with crystals impinging on it at the centre at angles varying from 5°-30°. He further observed parallel relationship between the advanced stage of disease and number of groups of patterns along with clarity of patterns.[15]

In the present study, with the method followed for blood collection, it is observed that sample could be preserved not only for 5-7 days but also up to 30 days. Similar procedure was used by Bessenich and Quadeer in their studies.[16,17] Pfeiffer has demonstrated positive result in 75% of cases while positive results were demonstrated in 91.1% cases when studied by Gruner. Quadeer in his study statistically stated that sensitivity of the new test procedure between 91.71 - 96.59%.[17,18] In the present study, according to statistical evidences this test was given 95% positivity in the subjects of the study group and 99% positivity in the subjects of control group.

Another aspect of dominant role of pattern formation force of blood must be acknowledged from the fact that the results of different workers under different laboratory conditions had the common feature of similar pattern formation in normal healthy individuals.[19-22] The same pattern in normal healthy individuals was also observed in the present study. Also, the characteristic pattern of crystals in some disease processes had shown some uniformity.

The meritorious works of Pfeiffer, Morris and Morris and Gruner have shown that the cupric chloride crystallization patterns differ in healthy and pathological conditions in human and animals.[10,15,20] The crystallization test gives different pattern in normal and pathological conditions was acknowledged since the epoch making discovery of Pfeiffer and confirmed subsequently by other workers.[15,16,23,24]

All the workers had observed non-specific, non-coordinated pattern with cupric chloride solution alone. Selawry and Koopmans reported thick textured needle like crystals either growing linearly or having side branching producing fork like arrangement. Quadeer had observed that completely dissimilar patterns in different dishes of cupric chloride alone. Selawry and Koopmans reported irregular side branching and branching at right angle from the main crystal. Such branching pattern could be observed in the present study.[16,17]

The whole plate presents orderly arrangement of radiating crystals from one area called as center of gravity. Similar pattern had been observed by other workers in their studies. The present study also confirms the results of most of the workers.[19-22]

Sabarth and Williams reported variations at the center of gravity. Instead of one center, two or more centers were observed in their study.[22] Similar findings were observed in the present study also. Sometimes these were in the form of wing formations, wings interspersed with spaces or group of radiating centers. Similar observations were made by Quadeer suggesting that instead of describing it as center of gravity, it should be termed as zone of gravity and the imaginary center of that area should be used to draw the vertical and horizontal axes to divide the field.[16,17]

As specified by Selawry, the basic radiation and tangential fans are both clearly seen when pattern formation was studied in case of benign conditions. Salawry had also reported less effect of benign growth on general condition of patients. However, she stated that the double tangential fan must be present to ascertain the benign conditions. "Hollow Glans" pattern in Blood Crystallization pattern is given all types of benign neoplasm irrespective of their origin from basic body tissues like epithelial, connective, muscular and nervous tissues.[25] In the present study only

2 of benign conditions had shown double tangential fan while in the remaining 11 cases showed hollow glans appearance as a confirmatory finding of benign conditions. Quadeer also obtained similar finding in his observation.[17] Typical hall mark of malignancy was shown to be transverse formation by Pfeiffer and subsequently by other workers.[17,21,22] Transverse formation was also observed in the present study in early as well as late cases of advanced cancer. A further insight into the formation of crystallization pattern in malignancy was given in detail by Selawry.[25]

CONCLUSION

It was observed in the present study that the pattern of crystallization was positive in early as well as late cases but in late cases it seems to be highly positive. Inference of the test shows that control group had shown 100% negativity while all cases of malignant as well as premalignant conditions showed 99% positivity. This indicates that test is 100% accurate and can be utilized as a screening method for the early detection of cancer.

From our study, we can conclude that crystallisation test is an interesting, valuable, economical, quick method for early detection of cancer. It was economical as the chemical itself is cheap and readily available. Also, short duration of the test makes it convenient to study more number of cases without much burden. Because of this, it can be used for mass screening programme of neoplastic conditions. This test has great value in detecting the presence of neoplastic changes in cases where the lesion is inaccessible to routine biopsy and other procedures.

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How to cite this article: Bali S, Marathe RR. Crystallization Test for Early Detection of Malignancy. Int Arch BioMed Clin Res. 2017;3(2):46-49.DOI:10.21276/iabcr.2017.3.2.10

Source of Support: Nil, **Conflict of Interest:** None