

## Study of Crystallization Pattern of $\text{CuCl}_2$ Solution in Benign and Premalignant Conditions at a Tertiary Care Teaching Hospital

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### ABSTRACT

**Introduction:** In majority cases, cancer 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. Crystallisation test has come to aid in this regard.

**Aim:** To study crystallisation pattern of cupric chloride solution in benign and premalignant conditions.

**Method:** 110 subjects were included in the 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 followed by detailed study of crystal patterns in benign and premalignant lesions.

**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. Hollow Glans formation characterizes the benign condition while hollow glans along with gap star formation characterizes the precancerous conditions.

**Conclusion:** The test is of great value and can be utilized as screening method in benign, cancerous and precancerous conditions.

**Keywords:** Crystallisation, Cupric Chloride, Precancerous, Benign, Blood.

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### Article History:

Received: 05-02-2017, Revised: 07-03-2017, Accepted: 28-03-2017

### Access this article online

Website: <a href="http://www.ijmrp.com">www.ijmrp.com</a>	Quick Response code 
DOI: 10.21276/ijmrp.2017.3.2.085	

### INTRODUCTION

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.<sup>1</sup> 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.<sup>2</sup> 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, inexpensive and confirmatory in nature.<sup>3-6</sup> Crystallisation test is one and may be used as an adjunct in the diagnosis of malignancy.<sup>7</sup>

When an organic extract is added to a solution of cupric chloride and the resultant mixture allowed is to crystallize, the crystals formed assume 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. A definite arrangement of crystals is produced when blood from normal

persons and from patients with malignancy is added to cupric chloride solution.<sup>8,9</sup> The test is based purely on physical phenomena and hence should be performed under controlled physical conditions.<sup>10</sup>

Kopaczewski (1933) suggested that the morphological forms of living beings are due to complicated arrangement of crystalline units as a whole. He 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.<sup>11</sup> The present study was conducted to study the crystallisation patterns of cupric chloride solution for benign and premalignant conditions.

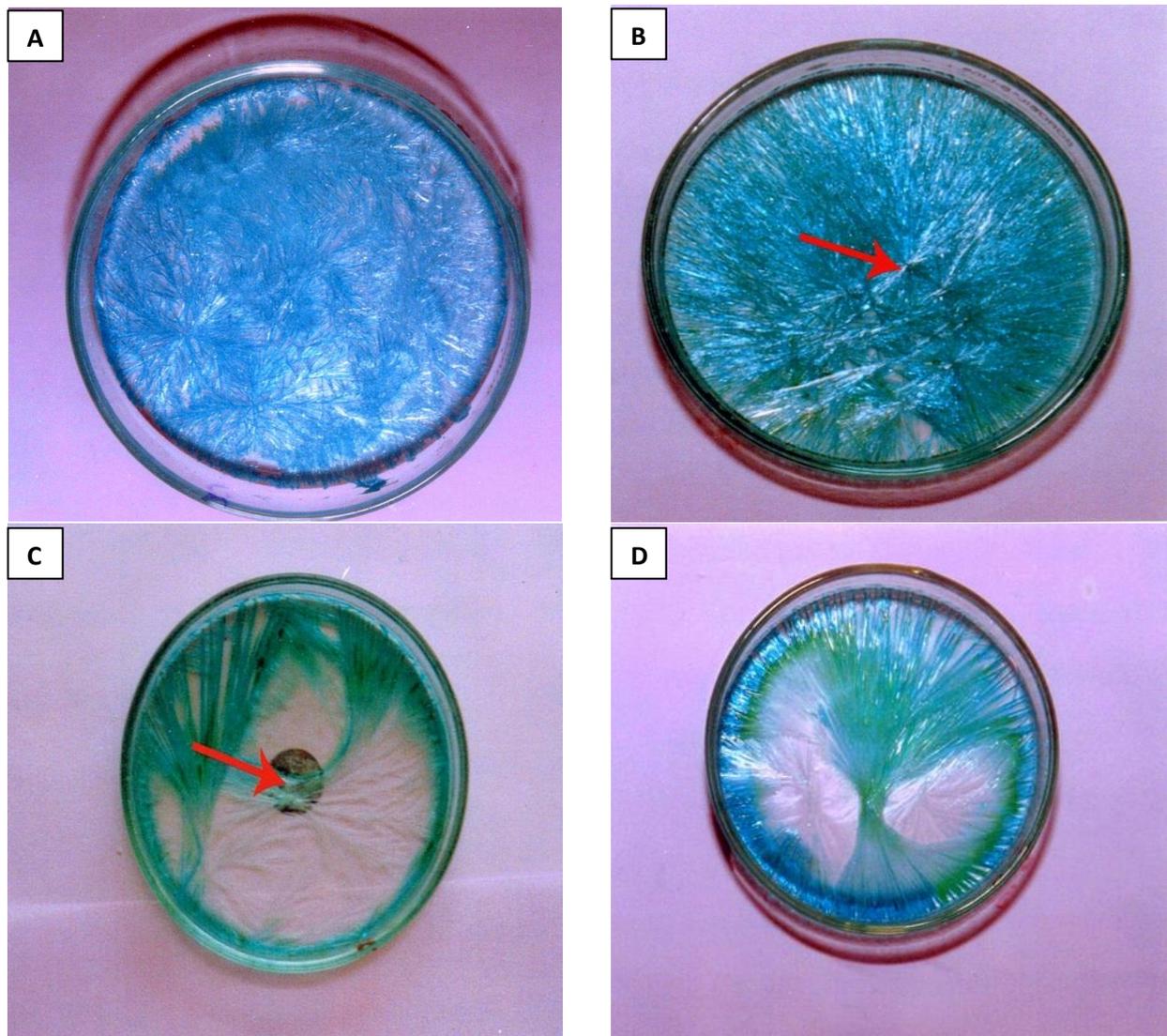
### MATERIAL AND METHODS

The cases with benign and premalignant conditions were studied irrespective of their age, sex and site of lesion constituting the study group. Control group included subjects who were

physiologically fit and belonged to a similar age range 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 malignant conditions. 47 malignant cases were excluded from the study and only the patterns for benign and premalignant lesions were studied in detail.

Eight drops of blood obtained by a finger prick were allowed to dry on Whatman filter paper No. 1. Dried drops of blood were then

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 carefully poured in the Petri dish covering the bottom of Petri dish. Five such Petri dishes were prepared for each subject and were placed in BOD incubator. Crystal formation was allowed for upto 24-30 hours at 32.7°C, without any disturbance. After crystallization was complete, the dishes were removed and examined. The near ideal Petri dishes were critically selected for each patient. They were then studied in the day light, photographed directly and observations were made.



**Figure 1: (A) Pattern of crystals from cupric chloride solution alone; (B) Pattern of cupric chloride in combination with human blood; (C) Crystallization pattern of blood from fibroadenoma of breast; (D) Crystallization pattern of blood from leucoplekia**

## RESULTS

The blood crystallization patterns are different in health and disease. In our study, the subjects were categorised according to the stage of neoplasm or the presence or absence of dysplastic features. The study group included patients suffering from precancerous, benign and malignant conditions of the respiratory system, nervous system, reproductive system, skin and oral cavity.

Crystallization pattern of cupric chloride solution when studied showed somewhat whorl formation [Fig 1 (A)]. 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 regularly arranged crystals which 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. This arrangement also highlighted the linear pattern of growth of the crystals with total absence of side branching and fanning of crystals [Fig 1 (B)]. All the crystals were arranged in one plane and there was no overlapping except near

the center of gravity. Due to eccentric position of the centre of gravity, complete field could be divided into two zones.

1. Zone with short radiations and
2. Zone with long radiations

The crystals were arranged in radiating fashion. In major part crystals were long and this part called as zone of long radiations, remaining part of petri dish called as zone of short radiations. Radiations usually did not reach up to periphery in the region of long needles and at the periphery additional narrow zone was present called as border zone. Centre of gravity can show slight variations in site and size.

Crystallization pattern when studied in benign conditions, it suggested a typical hollow glans pattern as seen in fibroadenoma of breast and seminoma testis. 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 1 (C)]. The hollow space has an acute angle at the apex and an obtuse angle at the base. Thus, it was roughly rhomboidal in shape, with in shallow lower half and deep upper half. The space was not completely empty but this traversed by the crystals of basic radiations.

Crystallization pattern in precancerous conditions showed complete absence of node formation, centre of gravity and fanning of crystals wherein crystals were arranged in such a way that it had a appearance of hollow glans formation or gap star formation and side branching of crystals or combination of both [Fig 1 (D)]. The crystallization pattern with hollow glans formation is present in most of the subjects of benign prostatic hypertrophy and fibroadenoma of breast.

## DISCUSSION

Crystallography is a special branch in science which deals with specific characteristics of crystals of organic and inorganic crystalline substances.<sup>12</sup> Glauber investigated the causes of crystallization, considering them to be of the nature of hitherto unfamiliar or "occult" forces.<sup>13</sup> Kopaczewski while discussing the physical origin of morphogenetic patterns in his treatise on colloid chemistry, gave an account of the history of the subject, quoting the work of Traube, Butschli, Tomlinson, Ord, Monnier and Vogt, etc.<sup>11</sup>

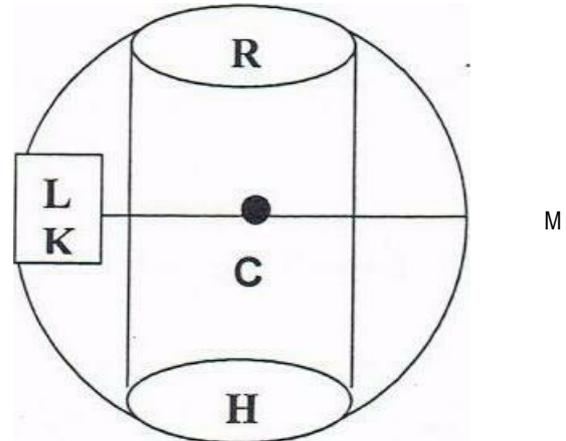
Various organic and inorganic substances were added to colloidal solutions and the resulting patterns were studied. Other workers stated that the test had diverse application in routine life to identify the adulterants in commercial (Jams and Jollies) or in biological tests for serum etc.<sup>14,15</sup>

In the present study, crystallization pattern from cupric chloride solution was found to be showing irregularly scattered radiations with overlapping of crystals along with bushy appearance. Some showed lack of co-ordination and whorl formation. 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°.<sup>16</sup>

Various authors observed eccentrically situated centre of gravity in the crystallization pattern.<sup>17</sup> Sabarth and Williams divided the whole pattern into four quadrants by two longitudinal axes-vertical and horizontal passing through and intersecting at centre of gravity (Fig 2). According to him head zone lies below center of gravity up to the periphery of glass plate and highlights the diseases of nervous system and special senses. Respiratory and

circulatory areas are located just above horizontal axis and the metabolic zone is beyond the former one. At the periphery of metabolic zone, on left side (4th quadrant) lies liver area and kidney area in just below metabolic zone. The border zone stands for limbs. If it is undeveloped, it represents physiological aging process.<sup>18</sup>

**Fig 2: Showing the distribution of system in the pattern of crystallization.**



- C - Centre of Gravity
- R - Respiratory plus cardiac system
- H - Nervous system and special senses
- L - Liver
- K - Kidney
- M - Metabolic Zone

A 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.<sup>15-18</sup> The same pattern in normal healthy individual is also observed in the present study.

The meritorious works of Pfeiffer, Morris and Morris and Gruner have undoubtedly shown that the cupric chloride crystallization patterns differ in healthy and pathological conditions in human and animals. Pfeiffer's test stresses the importance of physical and molecular forces in maintaining the integrity of molecules and the chemicals.<sup>15,16</sup> According to Gruner, these forces are in operation during crystallization process in pure state of chemical or when the chemical was mixed with some biological fluid.<sup>19</sup>

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. However, the exact factors responsible for the development of various patterns were not known and various theories were suggested. The crystallization pattern test advocated by Pfeiffer, Gruner, Selawry had the same base as observed in our present study.<sup>16,17,19</sup>

The addition of blood to cupric chloride solution essentially meant an addition of impurity. It was very difficult to pin point the actual substance acting as an impurity in case of blood as even one drop of blood contains number of vitally important substances. Koepf and Selawry (1962) reported extreme sensitivity of cupric chloride to proteins. The proteins in colloidal state play important role in the

pattern formation mechanism. During various pathological processes different pattern of cupric chloride crystal were observed by various workers which probably reflected on different types of proteins responsible for pattern formation.<sup>16,17</sup>

All the workers had observed non-specific, non-coordinated pattern with cupric chloride solution alone. Selawry and Koopmans have reported thick textured needle like crystals either growing linearly or having side branching producing fork like arrangement. In some, crystals were showing whorl like pattern and at other extreme numerous haphazardly arranged radiations were observed. An irregular side branching and branching at right angle from the main crystal was also noted.<sup>20</sup> Such branching pattern was also observed in the present study. Pattern of cupric chloride with healthy blood/ control group presented an orderly arrangement of radiating crystals from one area called as center of gravity. Similar pattern had been observed by other workers. Quadeer observed the crystallization pattern of cupric chloride in admixture with healthy blood presents striking contrast with that of cupric chloride alone.<sup>15-18,20</sup> As specified by Selawry the basic radiation and tangential fans are both clearly seen when benign conditions were studied for crystal pattern formation. Selawry also described the variations of patterns. Four different variations suggested by Selawry were as follows:

1. Simple broad hollow forms are rounded and bulge towards base. They are demarcated from surrounding by softly flowing double fan figure.
2. Simple medium hollow forms are uniformly oval and slightly narrow at the center or peripheral with double bundle of fan surrounding.
3. Narrow hollow have sharp angle and two narrow fans without double bundle at the base.
4. Occasionally double or multiple hollow forms are simultaneously based on double bundle. They are enclosed by several fans.<sup>17</sup>

"Hollow Glans" pattern in Blood Crystallization pattern is given in all types of benign neoplasm irrespective of their origin from basic body tissues like epithelial, connective, muscular and nervous tissues. The localization of benign neoplasm can however be done from the presence of pattern in definite zone of the organ system. This is accounted by Selawry as the less influence of benign neoplasm on the whole body as compare to that of malignant neoplasm.<sup>17</sup> 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.<sup>9</sup>

## CONCLUSION

It can be concluded that the crystallization test is significant. It helps in the diagnosis and is of great value in differentiating between the precancerous and benign conditions in cases where the lesion is inaccessible to routine biopsy and other procedures. It can be utilized as screening method in benign, cancerous and precancerous conditions.

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**Source of Support:** Nil. **Conflict of Interest:** None Declared.

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**Cite this article as:** Sangeeta Bali, R. R. Fulzele. Study of Crystallization Pattern of CuCl<sub>2</sub> Solution in Benign and Premalignant Conditions at a Tertiary Care Teaching Hospital. *Int J Med Res Prof*. 2017; 3(2):410-13. DOI:10.21276/ijmrp.2017.3.2.085