Role of Metal Salts in Blood Coagulation.

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***Abstract** : Thrombosis is a very common medical problem. The tendency for blood to clot too rapidly is an important diagnosis of serious pathological conditions like heart attack, stroke, coronary artery disease, deep vein thrombosis, and pulmonary embolism. The effect of metal ions on the blood clotting has been studied. A total number of 50 healthy volunteers in the age group of 18-35 with mean age 26.86 ± S.D 3.85 participated in the study. Addition of copper sulphate, silver nitrate and gold chloride in a range of concentration 100 – 500mg i.e. 10%, 20%, 30%, 40% and 50% and the in vitro effects of these salts on blood coagulation was studied. Clotting time was noted for each sample to form insoluble fibrin str respectively was done ands or a clot by Lee and White – Test Tube Inversion method, prothrombin and partial thromboplastin time was also measured. Copper sulphate caused a significant prolongation of clotting time (p value=0.000), prothrombin (p-value: 0.001) and partial thromboplastin times (p-value: 0.0001). This preliminary study shows that copper sulphate exhibits excellent anti coagulant property whereas silver nitrate and gold chloride did not alter the clotting time.*

INTRODUCTION

A thrombus or a blood clot is the final product of the blood coagulation which brings in hemostasis. Clotting is essential and it is life saving to arrest bleeding during trauma, surgery etc. it is well known when thrombus is not formed as in the case of hemophilic they can bleed to death. So that substantiates the necessity of clotting. However blood clots can also form abnormally obstructing the blood flow through the blood vessels causing myocardial infarction, brain stroke, gangrene of the extremities and other medical problems. Clotting disorders is a term used to describe a group of conditions in which there is an increased tendency for the blood to clot causing serious complications. **Thrombophilia is the sub specialty in hematology dealing with conditions involving thrombosis and bleeding diathesis. It** affects a large number of people around the world. Recent research shows that these disorders contribute significantly to morbidity and mortality in the United States. Each year, more than 600,000 Americans die from abnormal blood clots [1]. It is estimated that 60,000-100,000 Americans die of deep vein thrombosis or pulmonary embolism [2]. Metal ions are required for many critical functions in humans. Scarcity of some metal ions can lead to disease. Well-known examples include pernicious anemia resulting from iron deficiency, growth retardation arising from insufficient dietary zinc, and heart disease in infants owing to copper deficiency[3]. The effect of metal ions on the blood clotting is also diagnostically useful [4]. The principal object of the present invention is to provide a method to rapidly assess the overall coagulant properties of a sample of a blood with the metal ions, and subsequently measuring clotting time by standard methods. The measurement preferably includes the clotting time of a control sample of whole blood without the addition of metal ion, to which the clotting time of the sample containing the metal ion is compared. The preferred metal ions are copper, silver and gold for their biocompatibility, physiological and biochemical effects of these ions.

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Received on 23.03.15** **Accepted on 11.08.15

MATERIALS AND METHODS

The study was carried out by K.J. Hospital Research and Post Graduate Centre, Chennai. The study was cleared by institutional ethical committees and proper informed consent was obtained from the participants in the study. A total number of 50 healthy volunteers in the age group of 18 – 35 years participated in the study (mean age 26.86, SD ± 3.85). Under aseptic precaution vein puncture is done and blood samples were withdrawn for studying the clotting time of blood using various metal salts such as copper sulphate, silver nitrate and gold chloride. 3 ml of blood was drawn and 0.5 ml was transferred into four test tubes. Tube - 1: serves as control (Saline + Blood), Tube – II: Blood + Copper sulphate solution, Tube – III: Blood + Silver nitrate solution, Tube – IV: Blood + Gold Chloride solution. Five different concentrations (100 to 500mg i.e. 10%, 20%, 30%, 40% and 50% with the concentration being 400mmol/L, 801mmol/L, 1202mmol/L, 1602mmol/L and 2002mmol/L for copper sulphate; 589mmol/L, 1177mmol/L, 1766mmol/L, 2354mmol/L and 2943mmol/L for silver nitrate; 294mmol/L, 588mmol/L, 883mmol/L, 1177mmol/L and 1471mmol/L for gold chloride) of each salt were added in 1mL of saline and the contents were mixed thoroughly. 1 µL of the solution was extracted from the contents and added to 0.5ml of blood taken in four different test tubes. Clotting time was noted for each sample to form insoluble fibrin strands or a clot by Standard Lee and White – Test Tube Inversion method.

Lee and White – Test Tube Inversion Method

The whole blood clotting time is a rough measure of all intrinsic clotting factors in the absence of tissue factors. The aforementioned contents of each salts in test tube was kept in a water bath maintained at 37°c and the tubes were tilted for every 30 seconds to see whether the blood is flowing. This is repeated till the tube can be inverted without the blood flowing out and the time was noted.

Prothrombin Time (PT): The prothrombin time was developed to measure prothrombin (Factor II). The blood cells are separated from the liquid part of blood (plasma) by centrifugation. The PT test is performed by adding the plasma to source of Tissue Factor (e.g.: a protein, thromboplastin, from homogenized brain tissue) that converts prothrombin to thrombin. The mixture is then kept in a warm water bath at 37°C for one to two minutes. Calcium chloride is added to the mixture and allows clotting to start. The time takes for blood plasma to clot is noted. The time taken from the addition of calcium to the formation of the fibrin clot is known as the Prothrombin Time or PT and the normal amount of time it takes for blood plasma to clot is between 11 and 13.5 seconds.

Activated Partial Thromboplastin Time : This test is also used to evaluate the blood coagulation ability. The plasma is separated by centrifugation. Calcium and activating substances are added to the plasma to start the intrinsic pathway of the coagulation cascade. The substances added are kaolin (hydrated aluminum silicate) and cephalin. The partial thromboplastin time is measured in seconds. Normally, the sample will clot in 35 seconds.

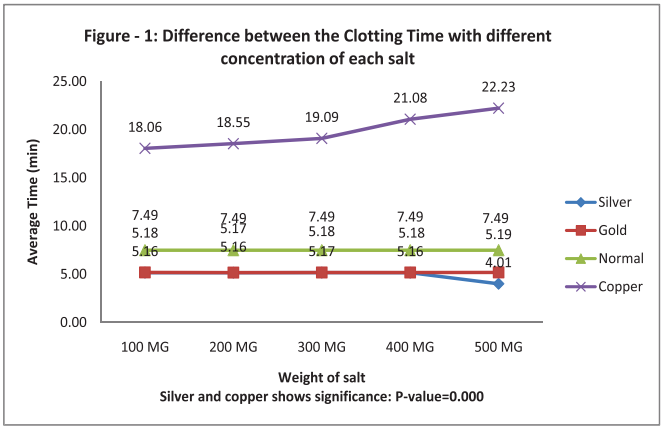
Statistical Analysis: Differences among the groups were determined using an analysis of variance model (TWO WAY ANOVA). Multiple range tests by Tukey-HSD procedure was employed to identify the significance at 5% level. A p- value less than 0.05 were considered as significant.

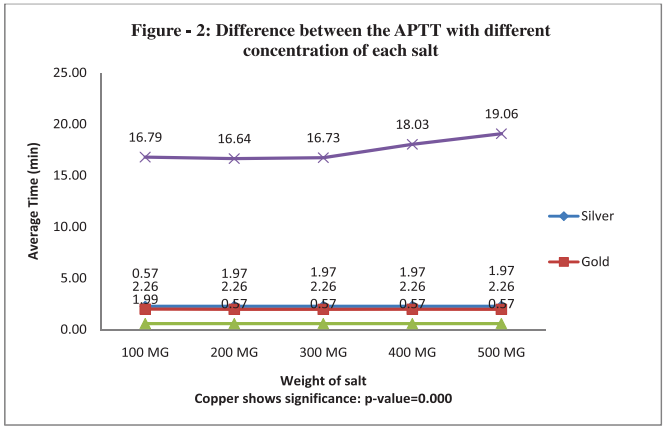
RESULTS

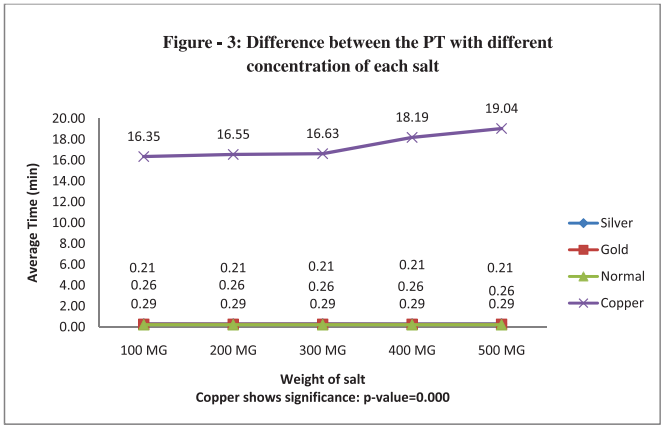
The effect of copper ions, silver ions and gold ions on the blood clotting time was determined. In the experiment that was performed in vitro, three metallic salts showed different levels on blood clotting time, Prothrombin Time and Activated Partial Thromboplastin Time. With the concentration of copper ions, there was a significant increase in blood CT, PT, APTT. Figure-1 shows the difference between the CT of varying concentration of each salt, silver and copper shows significance and the P-value=0.000. Gold ions did not show any significant change in the clotting time. Figure-2 shows the prolongation in the time of APTT with copper ions showing very high significance and the P-value=0.000 whereas silver and gold ions did not show any appreciable change in APTT. From figure-3 we can observe that the PT time significantly increased (p-value=0.000) with the addition of copper ions when compared with the silver and gold ions. Figure - 4: shows difference between the response of each salt with p-value: 0.000 for CT, p-value: 0.001 for PT and p-value: 0.0001 for APTT respectively. In vitro addition of copper sulphate (100 – 500 micrograms/ml) elicited an anticoagulant effect, though clot formation time was increased with all tested concentrations of copper sulphate when compared with silver nitrate and gold chloride.

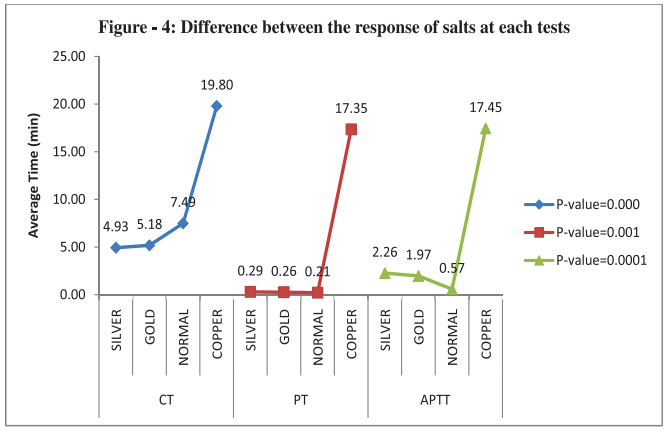
DISCUSSION

Copper is an essential nutrient and is one of a relatively small group of metallic elements which are essential to human health. These elements, along with amino and fatty acids as well as vitamins, are required for normal metabolic processes. Copper sulfate is an inorganic compound that combines sulfur with copper and has been used for over 50 years in nutritional supplements. It can kill bacteria, algae, roots, plants, snails, and fungi [5]. According to the world's scientific literature and the toxic and hazardous materials data files the results suggests that the









recommended daily allowance (RDA) of copper sulfate, which is 2 mg, has ever been associated with side effects or toxicity. Chierici R, et al studied influence on the trace element content of milk. In the study no adverse effects were seen when healthy lactating mothers were provided a dietary supplement containing 2 mg of copper sulphate [6]. Rock E, et al studied the effect of copper supplementation at doses up to 7 mg per day on red blood cell oxidizability and plasma antioxidants in healthy volunteers which caused no damage to red blood cells, and rather result in protection of red blood cells against oxidation [7]. Recent research in Egypt showed that feeding copper sulphate at 150 ppm improved daily weight gain and feed efficiency in broilers. However, copper carbonate or copper oxide did not show any response. Histological examination reveals that copper sulphate resulted in a healthier intestinal lining with a more absorptive surface. Another study on copper sulphate showed reduced cardiovascular risk factors that can promote atherosclerosis [8]. A data on the Extoxnet show that Copper sulphate has been shown to provide the most benefit and begins to be toxic at 750 mg - 375 times The RDA Dose.

Silver nitrate is a caustic chemical reagent and compound. It has got wide applications as an antiseptic. The use of silver nitrate for chronic wounds such as leg ulcers, as well as burns and acute wounds has already been described in literature [9]. There is currently conflicting evidence in the literature to support the use of silver nitrate due to some adverse effects. Use of this salt application reduces dramatically the size of large wounds, which eventually healed, avoiding the patients to undergo surgery [10]. However there are no reports supporting the evidences for use of silver nitrate as anti-coagulant agents.

Gold, in a variety of forms, has been used in medicine throughout the history of civilization. Gold complexes were introduced for the treatment for rheumatoid arthritis. The antimicrobial activity of the gold compounds has been investigated exhibiting broad spectrum activity against a range of organisms, with a small degree of specificity against the Gram positive organisms S. aureus and E. faecalis [11]. There are no reports available in the literature of using gold salts for exhibiting anti coagulant properties.

The PT measures the activity of the extrinsic and common pathways of coagulation and the **activated partial thromboplastin time** (**APTT**) is also used to evaluate the blood coagulation ability. While the prothrombin time (PT) tests the extrinsic pathway of the **coagulation cascade**, the partial thromboplastin time (PTT) tests the intrinsic pathway. The results of both tests together will allow for a collective assessment of the functioning of all **clotting factors**. In order to create a strong blood clot, a series of 12 plasma proteins, or coagulation "factors," act together to create a substance called fibrin. The prothrombin time (PT) will reveal deficiencies of factors II, V, VII, X or fibrinogen [12] while the activated partial thromboplastin time (APTT) indicate deficiencies of factors VIII, IX, XI, XII, fibrinogen deficiency, anti-factor antibodies like lupus anticoagulant [13]. This study shows that copper sulphate exhibits excellent anti coagulant property and it shows it's effective via intrinsic and extrinsic pathways whereas silver nitrate and gold chloride did not alter the clotting time.

CONCLUSION

This preliminary work showed that copper as an ion in solution shows a significant anticoagulant property when compared to gold and silver ions. This study indicates that copper ions have got effect in prolonging the clotting time through intrinsic and extrinsic pathways. Our future work is on synthesis and characterization of metal nanoparticles for implant applications.

ACKNOWLEDGEMENT

This work is supported by UGC-DAE-Consortium for Scientific Research, the project No. is CSR-KN/CSR-43/2012-13/735. We are thankful to Dr.Prakash Babu of Biochemistry Department of K. J. Research Foundation and Mrs.Princy George, Ms.Lavanya of K. J. Hospital for their invaluable help in this work.

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LITERATURE REVIEW

IMPACT OF DIALYSIS PRACTICE PATTERNS ON OUTCOMES IN ACUTE KIDNEY INJURY IN INTENSIVE CARE UNIT

Rajeev A Annigeri, Venkatappa Nandeesh, Ramanathan Karuniya, et.al. *Indian J. Crit Care Med 2016; 20:14-20*

Recent advances in dialysis therapy have made an impact on the clinical practice of renal replacement therapy (RRT) in acute kidney injury (AKI) in Intensive Care Unit (ICU). We studied the impact of RRT practice changes on outcomes in AKI in ICU over a period of 8 years. AKI patients requiring RRT in ICU referred to a nephrologist during two different periods (period-1: Between May 2004 and May 2007, n = 69; period-2: Between August 2008 and May 2011, n = 93) were studied. The major changes in the dialysis practice during the period-2, compared to period-1 were introduction of prolonged intermittent RRT (PIRRT), early dialysis for metabolic acidosis, early initiation of RRT for anuria and positive fluid balance and use of bicarbonate-based fluids for continuous RRT (CRRT) instead of lactate buffer. The primary study outcome was 28-day hospital mortality.The mean age was 53.8 ± 16.1 years and 72.6% were male. Introduction of PIRRT resulted in 37% reduction in utilization of CRRT during period-2 (from 85.5% to 53.7%). The overall mortality was high (68%) but was significantly reduced during period-2 compared to period-1 (59% vs. 79.7%, P = 0.006). Metabolic acidosis but not the mode of RRT, was the significant factor which influenced mortality. **Conclusions:** Adaption of PIRRT resulted in 37% reduction of utilization of CRRT. The mortality rate was significantly reduced during the period of adaption of PIRRT, possibly due to early initiation of RRT in the latter period for indications such as anuria and metabolic acidosis.