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Studies of the Effect of Old Age on Invitro Blood Clotting In Yenagoa Bayelsa State Nigeria.

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Abstract
Aging has been defined as "a progressive unfavourable loss of adaption, leading to increased vulnerability, decreased viability, and decreased life expectancy. Enablers of aging could be retarded as a result of proper medical intervention of the old aged. The aim of the research is to provide interventions that could also serve a retarder to aging and in the management of the aged. A total of 300 subjects were recruited into the study, 200 subjects constituted the aged defined based on 65 and above years. 100 subjects constituted the middle aged defined based on age range of 20-33 years. Samples were divided into two groups, group A constitutes the control (middle aged) and group B constitutes the aged. Platelets, serum calcium and clotting time were analyzed using WHO approved methods. The mean and SD of platelet count of group A 379±137 as compare to group B of 210±122. The CT for group A was 4.8±1.1 as compare to group B of 9.2±1.6. While serum calcium was 2.3±0.30 in the control as against the aged of 1.8±0.34. A statistical significances $p<0.05$ were observed for all the studied parameters. However, the platelets count and serum calcium had a decline in the aged as compared to the middle aged. Clotting time showed a significant increase. The decline in platelets count of the aged was attributed to decline in the thrombopoietin, senile production sites of platelets precursors and components. The prolonged CT observed for the aged was hinged on the decline in platelets count, coagulation factors and serum calcium concentration. Based on these finding, aged are advice not to expose themselves to injuries and cut. Also heath care providers should treat all injuries in the geriatrics as an emergency.
Keywords: Platelets, clotting time, geriatrics, calcium

INTRODUCTION

Most developed world countries have accepted the chronological age of 65 years as a definition of 'elderly' or older person, but like many westernized concepts, this does not adapt well to the situation in Africa. While this definition is somewhat arbitrary, it is many times associated with the age at which one can begin to receive pension benefits. At the moment, there is no United Nations standard numerical criterion, but the UN agreed cutoff is 60+ years to refer to the older population[1]. As far back as 1875, in Britain, the Friendly Societies Act, enacted the definition of old age as, "any age after 50", yet pension schemes mostly used age 60 or 65 years for eligibility. [1] .The more traditional African definitions of an elder or 'elderly' person correlate with the chronological ages of 50 to 65 years, depending on the setting, the region and the country. Adding to the difficulty of establishing a definition, actual birthdates are quite often unknown because many individuals in Africa do not have an official record of their birthdate [1,2]. Another difficulty in defining old age in Africa is the short life expectances variations. For this project, we shall adopt 65 years of age and older as the general definition of an older person.

Aging has been defined as "a progressive unfavourable loss of adaption, leading to increased vulnerability, decreased viability, and decreased life expectancy"[3]. In general, aging is associated with decreasing efficiency in maintenance of homeostasis, a decreased in cell water, a reduction in muscle mass, and a gradual decline in respiratory, cardiovascular, kidney, liver, immune system, neurological, and endocrine system functions. Aging is also associated with an accelerated increased incidence of many diseases, including diabetes, atherosclerosis, hypertention, and osteoporosis [3].

The study critically focused on three laboratory parameters pivotal in clotting evaluation; platelets counts, clotting time and serum calcium.

Platelets, or **thrombocytes** (from Greek θρόμβος, "clot" and κύτος, "cell"), are small, disk shaped clear cell fragments (i.e. cells that do not have a nucleus), 2–3 μm in diameter [4], which are derived from fragmentation of precursor megakaryocytes. The average lifespan of a platelet is normally just 5 to 9 days. Platelets are a natural source of growth factors. They circulate in the blood of mammals and are involved in hemostasis, leading to the

formation of blood clots. Stoppage of bleeding is the focal function of platelets. Its deficiency or excess is of worries to medical practice because of its negative health implications.

Clotting time is the physiological time required for a sample of blood to coagulate in vitro under standard conditions. In order for blood to clot, the enzyme thrombin must be generated from the plasma precursor prothrombin. Thrombin then converts soluble fibrinogen into insoluble fibrin. Generation of thrombin involves the sequential activation of a number of other plasma clotting factor, this process is also being assisted by Ca^{++} and by factors released by platelets and damaged tissues . The time taken for blood to clot mainly reflects the time required for the generation of thrombin in this manner. If the plasma concentration of prothrombin or of some of the other factors is low (or if the factor is absent, or functionally inactive), clotting time will be prolonged. The expected range for clotting time is 4-10 mins.T**hrombogenesis (Coagulation)** is the process by which blood forms clots. It is an important part of hemostasis, the cessation of blood loss from a damaged vessel is covered by a platelet and fibrin-containing clot to stop bleeding and begin repair of the damaged vessel. This is to aviod the collapsed of the circulatory system. Disorders of coagulation can lead to an increased risk of bleeding (hemorrhage) or obstructive clotting (thrombosis) [5]. Coagulation is highly conserved throughout biology; in all mammals, coagulation involves both a cellular (platelet) and a protein (coagulation factor) component [6]. Thrombogenesis could be accessed from two points of view. Prolonged thrombogenesis is a pointer to the fact that the apparatus for clotting is hampered, hence the propensity of severe bleeding and subsequent heart standstill is sure. Also, prompt, thrombogenesis is a pointer to easy coagulation, which is a factor in arteriosclerosis or stroke.

Calcium is one of the important cation found in the body. Over 99% of calcium in the body is part of the bone. The remaining 1% is mostly in the blood and other ECF [7]. Very little is in the cytosol of most cells. In fact, the concentration of ionized calcium in the blood is 5000 to 10,000 times higher than in the cardiac or smooth-muscle cells. Maintenance of this large gradient is vital to maintain the rapid inward flux of calcium ions. Calcium in blood is distributed among several forms. About 45% circulate as free calcium ions, 40% is bound to protein, mostly albumin, and 15% is bound to anions such as bicarbonate, citrate, phosphate, and lactate [7].

Decreased ionized calcium concentrations in blood can cause neuromuscular irritability, which may become clinically apparent as irregular muscle spasms, called tetany. Studies have shown that the rate of fall in ionized calcium initiates tetany as much as absolute concentration of ionized calcium [8]. Calcium is particularly important in a discussion of geriatrics because of its role in bone formation and strength. Total serum calcium shows some change with age and gender. Calcium tends to fall slightly in men, whereas they remain normal or slightly increased in women [7].

However, if the number of platelets is too high, blood clots can form (thrombosis), which may obstruct blood vessels and result in such events as a stroke, myocardial infarction, pulmonary embolism or the blockage of blood vessels to other parts of the body, such as the extremities of the arms or legs. An abnormality or disease of the platelets is called a thrombocytopathy [9], which could be either a low number of platelets (thrombocytopenia), a decrease in function of platelets (thrombasthenia), or an increase in the number of platelets (thrombocytosis). There are disorders that reduce the number of platelets, such as heparin-induced thrombocytopenia (HIT) or thrombotic thrombocytopenic purpura (TTP) that typically cause thromboses, or clots, instead of bleeding.

Platelet

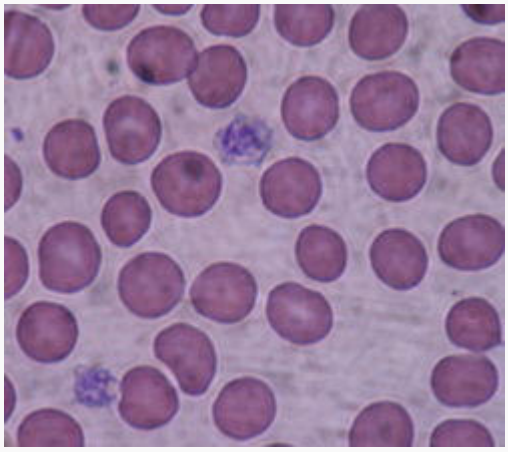

Image from a light microscope (40×) from a peripheral blood smear surrounded by red blood cells. One normal platelet can be seen in the upper left side of the image (purple) and is significantly smaller in size than the red blood cells (stained pink). Two giant platelets (stained purple) are also visible.

A normal platelet count in a healthy individual is between 150,000 and 450,000 per μL (microlitre) of blood ((150–450)×10^9/L)[10]. An adequate supply of circulating platelets is essential to maintain vascular integrity and to facilitate thrombus formation at sites of vascular injury. Given that platelets have a circulating lifespan of around 10 days, and that about one third of platelets are sequestered in the spleen at any time, approximately 100×10^9 of these small anucleate cells must be released from mature megakaryocytes into the circulation each day in order to maintain a normal platelet count. A constant balance is, therefore, required between thrombopoiesis, and platelet consumption and senescence. Thrombopoietin is the primary humoral regulator of megakaryocyte differentiation and platelet number under steady state conditions[11]. It is synthesized in the liver and kidney and mediates its effects through its receptor c-Mpl which is present on megakaryocyte and platelet membranes [11]. Levels of thrombopoietin are controlled via binding to, and internalization into, cells expressing the receptor. When platelets and megakaryocytes are decreased in number, less thrombopoietin is removed from plasma, and the thrombopoietin level rises, while when platelet numbers increase, more thrombopoietin is cleared from the plasma and the thrombopoietin level falls again [12]. In addition to the thrombopoietin (THPO) and c-Mpl (MPL) genes, a large number of genes encoding membrane glycoproteins, cytoskeletal components and proteins involved in transcription, cell cycle regulation and signaling have been demonstrated to participate in the differentiation of pluripotent stem cells to mature platelet-shedding megakaryocytes [13].

An explanation for the age-dependent decline in platelet count observed by Biino et al. and others is still awaited [14]. It has been suggested that it may be due to reduced hematopoietic stem cell reserve in aging individuals[15]. It is also possible that the age-dependent decline in platelet number reflects epigenetic changes in the megakaryocyte genome, such as hypomethylation of genes that determine platelet count or changes in histone acetylation, which lead to differences in gene expression as we get older. The seasonal factors that in one longitudinal study accounted for 2% of the overall variance in platelet count, and caused a peak in platelet count during autumn and winter months, also remain to be clarified, though it is possible that this reflects the increased prevalence of viral infections during the winter months, since infection has been established to increase platelet count [16]. A work carried out by [17] showed platelets variations in aging: platelet count was similar in men and women until the age of 14, but subsequently women had steadily more platelets than men. The number of platelets decreases quickly in childhood, stabilizes in adulthood, and further decreases in oldness. The final result of this phenomenon is that platelet count in old age was reduced by 35% in men and by 25% in women compared with early infancy. Based on these findings, we estimated reference intervals for platelet count ×10^9/L in children (176–452), adult men (141–362), adult women (156–405), old men (122–350) and, old women (140–

379). Clotting time as well known is determined by the quantum of platelets in the blood. Hence a decrease platelet counts can prolong clotting time and increased platelet counts can decrease clotting time.

The aim of this research work is to compare platelet counts and clotting time of the middle aged adults to that of the aged (geriatrics) so as to design a separate reference range in Bayelsa State, Nigeria, which is lacking at the time of the research work. Also, this research will provide an appropriate treatment and preventive regimes for the management of the geriatrics in course of extensive injury sustenance.

2.0 MATERIALS AND METHODS

2.1 Study Location

This study was conducted at the Departments of Haematology and Blood transfusion of the Federal Medical Center, Yenagoa, Bayelsa State. Bayelsa state is located within Latitude 4^0 15^1 North and Latitude 5^0 and 23^1 South. It is also within longitude 5^0 22^1 West and 6^0 45^1 East. It is bounded by Delta State on the North, Rivers State on the East and the Atlantic Ocean on the Western and Southern parts. According to the 2006 census figures, Bayelsa has a population of about 1.7 million people.

2.2 Study subjects

Three hundred (300) subjects were utilized in this study. The subjects were divided into two groups. The group A is made up of the 100 apparently healthy subjects within the age bracket of 20 to 36 years which constitute the control group. Group B is constituted of 200 elderly subjects within the age range of 65 to 76 years

2.3 Ethical Clearance

Ethical approval was granted by the department involved in the study. Participation was voluntary and informed consent was obtained as verbal or written depending on the literacy level of individual participants.

2.4 Sample collection

In total, 300 blood samples were collected from 300 subjects by venepunture. The blood was immediately ejected into EDTA and plain containers. Samples were assayed immediately after sample collection.

3.5 Analysis

Platelets count

Platelets were analyzed using automated systemex analyzer XS1000i.

Serum calcium

Serum was assayed using O-Cresophthalein Complexone Method, without deproteinization (colorimetric test for calcium).

Principle: In an alkaline medium, calcium ions form a complex with O-Cresophthalein Complexone.

The SOP prepared by Randox reagent Ltd was used as stipulated in the manual of operation.

Clotting time

2ml of venous whole blood was dispensed into a dry clean small glass tube and left undisturbed. It was then observed for clotting after 20 minutes by tilting the tube.

2.6 Statistical analysis

Data were analyzed with SPSS program (SPSS Inc., Chicago, IL, USA; Version 15) and expressed as mean ±SE. Student t-test was used for comparing values of the two groups. Percentages and pictorial expression were also used for data presentation.

RESULTS

Table 1.1: A comparison of mean±sd of the platelets count, clotting time and serum calcium between control and in the aged.

Parameters	Reference Range	Control mean±sd Group A (n=100)	Aged mean±sd Group B (n=200)	p-value	comment
Age (yr)		26±6	70±6	p<0.05	s
Platelets (10^9/L)	140-400	379±137	210±122	p<0.05	s
CLOTTING TIME (MINUTES)	5-8	4.8±1.1	9.2±1.6	p<0.05	s
SERUM CALCIUM (mmol/l)	2.2-2.7	2.3±0.30	1.8±0.34	p>0.05	s

S-significant

Table 1.1 shows a significant decrease in platelets count, clotting time and serum calcium of group B as against group A.

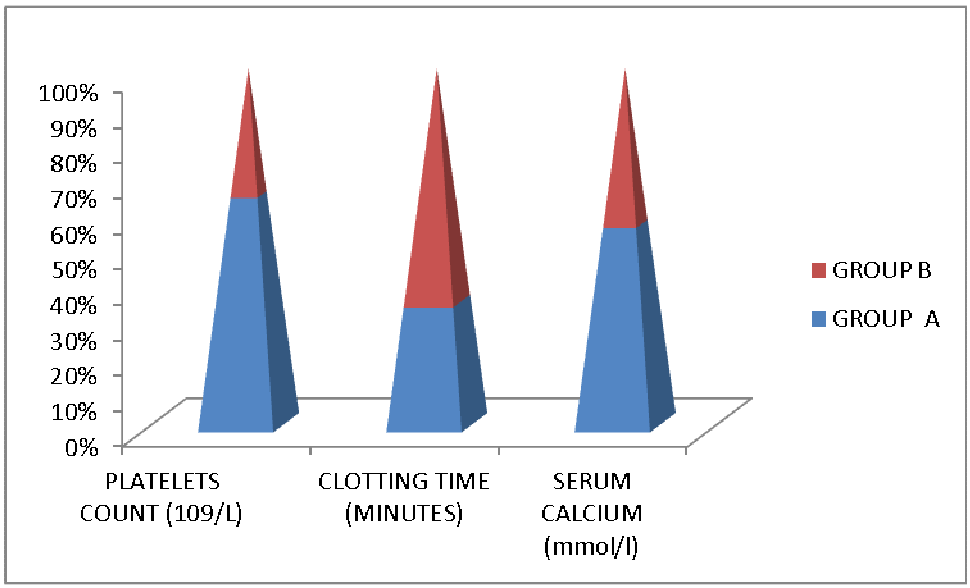

FIGURE 1.0. A comparison of mean±sd of the platelets count, clotting time and serum calcium between control and in the aged.

Discussion

The research carried out showed a decrease in platelets count in the old aged as compared to the controls (middle aged). Also, the clotting time of the geriatrics was almost two folds greater than that observed for the middle aged. As for the serum calcium, a slightly significant decrease was observed for the old aged. The core function of platelets is the formation of mechanical plugs during the normal haemostatic response to vascular injury. A decrease platelets count or inactivity will alter the body response to prompt wound healing processing. This depicts that in the elderly, formation of platelets plug to stop bleeding will be delayed, and hence more blood loss. This work further confirmed a longitudinal cohort studies carried out by [18] on platelets counts in the elderly.

The fall of platelet counts in the aged could be attributed to two schools of thoughts. Firstly, the decline could be credited to decrease in the production or inactivity of thrombopoietin. The hormone thrombopoietin is involved

in the balance between platelets consumption and production. Thrombopoietin is the primary humoral regulator of megakaryocyte differentiation and platelet number under steady state conditions [11]. It is synthesized in the liver and kidney and mediates its effects through its receptor c-Mpl which is present on megakaryocyte and platelet membranes[11]. Levels of thrombopoietin are controlled via binding to, and internalization into, cells expressing the receptor. When platelets and megakaryocytes are decreased in number, less thrombopoietin is removed from plasma, and the thrombopoietin level rises, while when platelet numbers increase, more thrombopoietin is cleared from the plasma and the thrombopoietin level falls again. The second school of thought is based on the aging status of the sources of platelets production. Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes [19]. The precursor of the megakaryocytes-arises by a process of differentiation from the haemopoietic stem cell [19]. The processes involved in the synthesis of platelets precursor in the stem cells is complex and is possible that one or two factors in the cascade is affected by aging, hence the fall of platelets. These two schools of thoughts are glaring possibilities that are responsible for the fall of platelets in the aged.

However, clotting time is a complex process being regulated by platelets count and activity. A fall in platelets will definitely lead to a prolonged clotting time. The prolonged clotting time observed in the aged is due to the decrease in platelets counts and its inactivity.

Also, clotting time is dependent on coagulation. Blood coagulation involves a biological amplification system in which relatively few initiation substances sequentially activate by proteolysis a cascade of circulating precursor proteins (the coagulation factor enzyme) which culminates in the generation of thrombin; this, in turn, converts soluble plasma fibrinogen into fibrin [19]. Fibrin enmeshes the platelet aggregates at the sites of vascular injury and converts the unstable primary platelets plugs to firm, definitive and stable haemostatic plugs [19]. Coagulation cascade is a complex process and the prolonged clotting time could also be credited to the effect of geriatrics on the coagulation factors that help in arresting bleeding promptly. Another cause of prolonged clotting time is in the role of calcium in fibrin formation. Calcium is a cation that plays significant role in clotting. It serves a cofactor in the clotting enzymes cascade. Calcium and phospholipid (a platelet membrane constituent) are required for the tenase and prothrombinase complexes to function. Calcium mediates the binding of the complexes via the terminal gamma-carboxy residues on FXa and FIXa to the phospholipid surfaces expressed by platelets, as well as procoagulant microparticles or microvesicles shed from them. The decrease in serum ionized and cytosolic free calcium will definitely prolong the time for clotting, hence leading to more bleeding in the aged as compared to the middle aged. The decline in serum calcium among the aged is a proof that old age could cause prolonged coagulation.

CONCLUSION

It is a known scientific truth that platelets central function is maintaining integrity of the vascular system. The study has clearly elucidated that aging has the propensity of decreasing platelet counts which leads to prolonged clotting time. The fall in serum calcium in the aged is another pointer to the fact that old age is a factor in prolonged invitro coagulation. The aged should abstained for activities that will make them vulnerable to injury, as bleeding arrest is more difficult and complex due to the decreased in plaetelets counts and decreased serum calcium. Also, care providers should be prompt in arresting injuries of the aged as blood loss is more severe. Finally, scientists should critically study the other factors directly involved in prolonged clotting time as it will be useful in arresting bleeding in the elderly promptly.

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