

A Comparative Study Of Reticulocyte Count In Healthy Young Adult And Elderly Age Group Subjects

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Abstract: Introduction: Aging process has been viewed as progressive decline in physiological process necessary for maintenance of a constant milieu interior and death as the ultimate failure to sustain homeostasis. Reticulocytes are juvenile red cell. It is flat non-nucleated disc shaped, slightly larger than mature erythrocyte. The number of reticulocyte present in the peripheral blood is a fairly accurate reflection of erythropoietic activity of bonemarrow. Normal values in young adult- 0.5 to 2.5% of total RBC count. The present study was conducted to find out changes in the reticulocyte count in the elderly subject. Aims: to uncover the effect of aging on reticulocyte count. Material and method: 90 subjects were divided into two groups, normal control group (n=30, mean age 35.83yrs) and elderly group (n=60, mean age 62.55yrs). Reticulocyte counts were done by new Methylene Blue method. Result & Observations: significant decrease in reticulocyte count was observed in elderly group as compare to normal control group. Conclusion: our data suggests that aging may alter haematological parameters including reticulocyte count. [Jain P et al NJIRM 2013; 4(2) : 118-122]

Key Words: Aging, Reticulocyte, Elderly

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Introduction: Aging is described as the process that reduces the number of healthy cells in the body; therefore, the body loses its ability to respond to a challenge to maintain homeostasis.^{1, 2.}

Aging process has been viewed as progressive decline in physiological process necessary for maintenance of a constant milieu interior and death as the ultimate failure to sustain homeostasis. Reticulocytes are juvenile red cell. It is flat non-nucleated disc shaped, slightly larger than mature erythrocyte. The number of reticulocyte present in the peripheral blood is a fairly accurate reflection of erythropoietic activity of bonemarrow. Normal values in young adult- 0.5 to 2.5% of total RBC count. The present study was conducted to find out changes in the reticulocyte count in the elderly subject.

Genetic damage is almost certainly a (or probably the) central cause of aging." Aging process can be viewed as an integral aspect of the continuous development of an organism. The study of the physical and physiological changes incident to old age is called gerontology.^{2,3} Studies of hematological aging in humans generally begin with investigations of population samples, representative for the background population

regarding age, sex, and health status, socio-economic, environmental and hereditary factors. The increasing interest in the aging human is partly due to the fact that the fraction of elderly in most countries is steadily increasing, i.e. the "geriatric boom".^{4, 5} Here an attempt has been made to review the comparative hematological changes in young adult and age subject.

Hematological Changes In Aging: The study of blood and blood cells (hematological parameters) is the object of continuing intensive research of the hematopoietic system to the functional integrity in the elderly. Various researchers have reported an association of hemorheological, hematological and metabolic changes with human aging.^{4,5,6}

Aims & Objectives: To uncover the effect of aging on reticulocyte count.

Material & Methods: The present study was carried out at Department of Physiology, Shri M. P. Shah Medical College and Guru Govind Singh Hospital, Jamnagar. Total 90 healthy subjects were selected. Out of which 30 healthy subjects were young subjects (normal control group) and 60

healthy subjects were elderly subjects. Complete detailed history of each subject including age, sex, height and specific symptoms (if any) were noted as shown in proforma. After detail history, general and systemic examinations were done thoroughly. Blood collection: Blood is drawn from a vein, with all antiseptic precautions, collected in an airtight vial or a syringe.

Reticulocyte Count: Reticulocyte count was done by new Methylene Blue method. Reticulocytes are immature RBCs that contain remnant cytoplasmic ribonucleic acid (RNA) and organelles such as mitochondria and ribosomes. Reticulocytes are visualized by staining with vital dyes that precipitate the RBC and organelles to form microscopically visible dark-blue clusters and filaments (reticulum). An erythrocyte still possessing RNA is referred to as a reticulocyte.

Equipment: 1. Liquid new methylene blue solution. 2. Slides. 3. Microscope. 4. Test tubes.

Procedure: Preparation of smears: Add 5 drops of new methylene blue solution to 5 drops of thoroughly mixed EDTA anticoagulated blood in a test tube, mix the contents by gently shaking and allow to incubate at room temperature for a minimum of 10 minutes. At the end of 10 minutes, gently mix the blood/stain solution. After that place a drop of the mixture on the slides and make a peripheral acceptable smear not too thick or thin and dried it.

Manual method of counting: Place the first slide on the microscope stage and using the low power objective (10x), an area is find out in the thin portion of the smear in which the red cells are evenly distributed and are not touching each other. Carefully change the oil immersion objective (100x) and further locate an area in which there are approximately 100 red cells per oil immersion field. I did this by finding a field where the cells are evenly distributed and mentally divide the field into 4 quadrants. Count all cells that contain a blue-staining filament or at least 2 or more

discrete blue aggregates of reticulum in the erythrocyte.

Field no.	No. of reticulocytes	No. of RBC	Total no. of cells
1			
2			
3			
4			

Calculation

Reticulocyte count (%) =	No. of reticulocytes x 100
	Total no. of cells

Precautions: 1. A refractile appearance of erythrocytes should not be confused with reticulocytes. 2. Filtration of the stain is necessary when precipitated material is present which can resemble a reticulocyte. 3. Erythrocyte inclusions should not be mistaken for reticulocytes. 4. Falsely decreased reticulocyte counts can result from understaining the blood with new methylene blue. Be sure the stain/blood mixture incubates the full 10 minutes.

Data Analysis: 1) Mean 2) Standard deviation 3) Standard error of difference between two means 4) Calculation of 't' : Then probability is calculated from 't' tables.

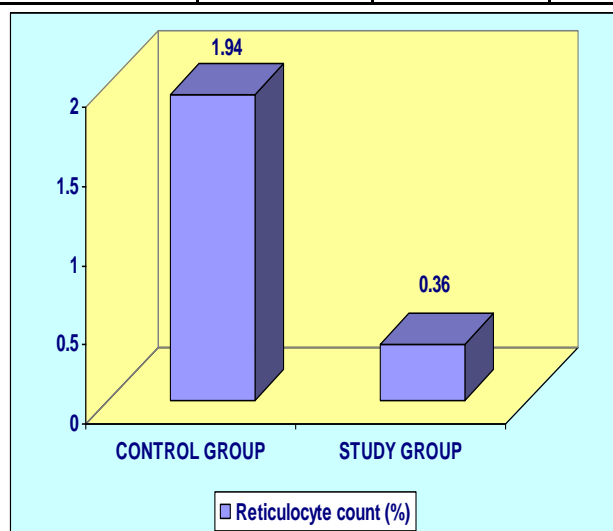
Result & Observations: In our study, 90 cases were studied. The control group comprised of the subject of the age group ranging from 20 – 50 years. The study group included subjects above the age of 50 years as a landmark, to access the effect of aging on reticulocyte count. There is Significant decrease (P= .005) in reticulocyte count was observed in elderly group as compare to normal control group.

Discussion: Vellar O. D. et al. (1967) observed decrease in hemoglobin, hematocrit, MCHC values and reticulocyte count in old men and women.

Giorno R. et al. (1980) observed significant decrease in the following hematologic parameters

Mean age and reticulocyte count in control group and in study group.

PARAMETER	CONTROL GROUP (Mean \pm SD)	STUDY GROUP (Mean \pm SD)	Z value
Age (in years)	35.83 \pm 8.90	64.86 \pm 9.72	-
Reticulocyte count (%)	1.94 \pm 0.44	0.36 \pm 0.10	171.62



erythrocyte count, hemoglobin, hematocrit, mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC).^{15,16,17} Significant age differences, which were most striking for leukocyte count, erythrocyte count, MCV and MCH were detected. Van Oosterhout EC. et al. (1989) reported the significant decrease in haemoglobin values (7.4-10.5 mmol/l) and erythrocyte counts (3.8-5.5 \times 10¹²/l).¹⁶ Ohhara Y. et al. (1994) assessed hematological parameters in the elderly and reported that red blood cell count (RBC), hemoglobin level (Hb), hematocrit value (Hct) showed a significant decrease in the older group.¹⁸ No significant age-related changes were observed in WBC. A significant decline with age was observed in both male and female MCV values. On the contrary, from a cross-sectional standpoint, the MCV values in the older female group were

higher than those in the younger group.^{15,16,17,18} Suwannuruks R. et al. (1997) reported advancing age is associated with the decline of hemoglobin, red blood cell count, hematocrit and other parameters. This study determined the hematologic parameters of Thai subjects aged over 50 years by using a fully automated cell counter. The MCV of both sexes showed an upward trend at age over 70. The red blood cell count and Hb levels in males were higher than females.¹⁹

These however, were not significant alterations. Takubo T. et al. (2000)¹⁷ observed red blood cell count, Hb concentration and hematocrit for the healthy aged showed the tendency of moderate decreasing with aging.¹⁸ These phenomena become more obvious by dividing the healthy aged into three groups 65-74 years, 75-84 years and over 85 years. The mean corpuscular volume(MCV) for the healthy aged showed the tendency of increasing with aging, while mean corpuscular Hb and mean corpuscular hemoglobin concentration for the healthy aged showed tendency of decreasing with aging. No changes for the healthy aged were observed in the leukocyte differential, white blood cell count and platelet count with aging.^{15,16,17,18,19} Martin H. et al. (2001) observed decrease in hemoglobin concentration in aged people.

Also reported that advancing age is associated with four indices shows significant differences (MCV increasing, MCH decreasing, MCHC decreasing, Red Cell Distribution Width increasing).²² In the older group, the iron level and the transferrin saturation were also significantly lower. In women, erythrocytes and the hematocrit are significantly higher in the older group whereas the indices MCH and MCHC are lower and the RDW increases. He also reported decrease in reticulocyte count in aged people.²² Blain H. et al. (2001) reported red blood cell count, hemoglobin concentration and hematocrit for the healthy aged showed the tendency of decreasing with aging. Mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) also decreases in aged. Reticulocyte count decreased in men and women aged 65 years and over.^{21,22}

The present study included 30 cases of young adult subjects as a control group and 60 cases of elderly subjects as a experimental group.

The mean age (in years) of young adult subjects was 35.83 ± 8.90 and for elderly subjects was 72.13 ± 7.25 . General and systemic examinations of the all subjects were normal. Various investigators have studied age related changes in hematological parameters including reticulocyte count by different methods. Regardless of the methods used, the conclusion is that with advancing age hematological parameters decreases.

The available literature on Indian subjects is scanty. So we have compared our observations with those available from western countries.

Both the control group and study group were compared in terms of age and reticulocyte count. The mean reticulocyte count and S.D. of control group is 1.94 ± 0.44 , whereas in study group it is 0.36 ± 0.10 . This is showing significant decrease in reticulocyte count in study group as compare to normal control group. Reticulocyte count significantly decreased in elderly group. It does not appear to be linked to a decrease in erythropoietin production with aging. Exact mechanism is not known, Might be due to many causes but most commonly due to ineffective erythropoiesis.

The reticulocyte count is significantly decreased in elderly subjects. These findings are comparable with the observations of other workers. Blain H. et al. (2001) observed significant decrease in reticulocyte count in elderly subject and this is similar with present study.²¹ Martin H. et al. (2001) found significant decrease in reticulocyte count in older group and this is also similar with present study. 22

Conclusion: This is study of total 90 cases, 30 are in control group and 60 are in study group. All are healthy. Our data suggest **significant decrease** in reticulocyte count in elderly subject as compare to young adult. Our data suggests that aging may alter various haematological parameters including reticulocyte count. The result shows a significant

decrease in Reticulocyte count is a fairly accurate reflection of erythropoietic activity of bonemarrow. Further studies with large sample size and long follow up may reveal more information.

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