



## AGING AFFECTS THE NUMBER OF WBC AND ITS SUBSETS IN A PAKISTANI COHORT OF YOUNG AND ELDERLY WOMEN

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### ABSTRACT

Aging is a universal phenomenon with an inconspicuous decline in nutrition and immune functions. The present study was undertaken to establish the effect of aging on selected cells of immune function in a cohort of young and elderly female in Pakistan. A sample of 60 (30 each young and elderly) generally healthy women participated in the study. Anthropometrics (weight, height, WC) were measured and BMI and WHR were calculated following standard procedure. Dietary intake was assessed by 24-hr dietary recall. Fresh blood samples were used for WBC and its differential counts. The results demonstrated age-related changes in the number of WBC and its subsets. The number of platelets decreased significantly with increasing age ( $p > 0.0001$ ). The number of WBC and its subsets (basophils and neutrophils) decreased with increasing age. However, these trends were statistically non-significant ( $p$ , for all trends  $> 0.05$ ). Monocytes significantly increased with increasing age ( $p = 0.0096$ ), while lymphocytes decreased with age but only with a borderline statistical significance ( $p = 0.051$ ). In conclusion, normal aging has a significant effect on the number of platelets, WBC and its fractional counts. Most of the cells decrease with increasing age. These changes may have important clinical implications and therefore, further investigations are needed.

**Keywords:** aging, immunity, blood cells, nutritional status.

### INTRODUCTION

It is well demonstrated that immune-competence declines with age (Alam *et al.*, 2012a, 2012b). With normal aging the immune system begins to lose some of its functions and cannot respond as quickly or efficiently to stimuli. Age related changes in the immune system have been observed at all levels ranging from chemical changes within the cells, to differences in the kinds of proteins found on the cells surface, and even to alterations in the entire organs. Some of these changes may seem trivial, but when all of the changes are added up, they radically affect the overall health of the particular individual. Besides other factors, no of cells of immune system is also with aging. In the same way, the activity of leukocytes (macrophages, monocytes, neutrophils and eosinophils) are reduced in the elderly, although there is very little data available on the effects of aging on these cells types.

The primary function of white blood cells, or leukocytes, is to fight infection (Alam *et al.*, 2012a). There are several types of white blood cells and each has its own role in fighting bacterial, viral, fungal, and parasitic infections. Types of white blood cells most important for helping protect the body from infection and foreign cells include, neutrophils, eosinophils, lymphocytes, monocytes and granulocytes.

The number of elderly is tremendously increasing in Pakistan (Alam I, 2013; Pakistan Demographic Survey, 2003) and hence maintaining good health and well-being of this particular age group is becoming even more important. Besides a great number of other health risks associated with old age, this population is potentially the most vulnerable group for malnutrition (Alam *et al.*, 2011a; Alam & Bangash, 2010; WHO, 2006). Similar to other developing countries, Pakistan can be expected to

experience the impact of an increasingly ageing population over the next few decades (Pakistan Demographic Survey, 2003; Alam *et al.*, 2012b; Alam *et al.*, 2012c), with a steady rise in the average life expectancy from 59.1 years in 1991 to 65 years in 2002. This quite sudden demographic shift can be very challenging in terms of health. The present study was, therefore, undertaken to investigate the effects of natural aging and nutritional status on the number of WBC and its subtypes in a sample of elderly women.

### METHODS

#### Sample location and selection

The study was conducted in one of Union Council health facilities of Malakand Agency, Khyber Pakhtunkhwa (KPK) province of Pakistan. A convenience sample of 60 women was selected. The mean age of young and old women were, (26.5; 58.8yrs respectively (range: 23.5-29.5yrs; 50.3-66.6yrs respectively). The inclusion criteria were: all subjects were healthy, non-diabetic, with no cardiovascular diseases (CVD), and without any recent past history of infection.

#### Anthropometrics and blood sample collection

General Anthropometrics were measured: weight was measured to the nearest 0.01Kg on a bathroom scale. Height was measured to the nearest 0.01 cm using an ordinary non-stretchable tap. Dietary intake was assessed using 24 hr dietary recall. Subjects were asked what they had eaten during the previous day starting from the morning breakfast till the last meal before going to bed (Alam *et al.*, 2012b; Alam *et al.*, 2012c). For enumeration of WBC, 3.0 ml of blood was withdrawn from each



healthy donor under complete aseptic conditions. They were dispensed into a tubes containing EDTA as anticoagulant for performing complete blood count (Alam *et al.*, 2011b).

Written informed consents were obtained from all the participants before the start of study.

### Statistical analysis

All anthropometric measurements, data on nutrient intake and immune cells were made in duplicate and the means of paired values were used in the analyses. The data were statistically analyzed using SAS (Version 7.0. SAS, USA). For comparison of means, two-sample t-test was performed to compare the variables of interest of the two groups. A correlation analysis was also performed to calculate the coefficients of correlation and to establish associations between anthropometric measurements, parameters of body composition, nutrient intake and immune cells.

### RESULTS

Table-1 shows the general characteristics of the study subjects. The age of the elderly and young ranged from 50.3 - 66.6 (Mean: 58.8 yrs) and 23.5 - 29.5 yrs (Mean: 26.5 yrs), respectively. There was non-significant difference in the mean values of weight, BMI, WC and % BF between the elderly and young (*p*, for all trends >0.05). Mean weight of elderly (66.0 Kg; Range: 49.7 - 84.2 Kg) was not different from the mean weight of young (63.5 Kg; Range: 48.8 -88.8 Kg). BMI, WC and % BF of the elderly and young ranged from 16.1 - 31.4 Kg/m<sup>2</sup>; 56.0 - 104.4 cm; 7.8 - 37.4 % and 17.3 - 29.5 Kg/m<sup>2</sup>; 56.3 - 104.2 cm, 4.7 - 36.2 %, respectively. Table also shows the mean intake of selected nutrients (energy and protein). There was non-significant difference in the mean values of protein and energy intake between the elderly and young (*p*, for all trends >0.05). Energy intake ranged from 1535 - 2895 Kcal/day (elderly) and from 1596 - 3185 Kcal/day (young). Protein intake ranged from 22.9 - 53.3 g/day (elderly) and from 29.6 - 54.5 g/day (young).

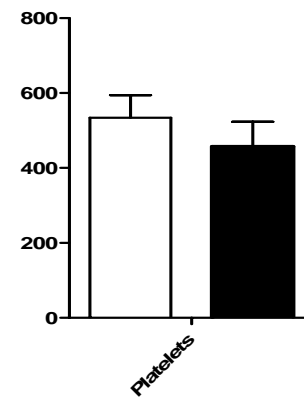
**Table-1.** Age, selected anthropometrics and nutrient intake of the subjects.

	Old	Young	p-value
<b>Age and anthropometrics</b>			
Age (years)	58.8±3.4	26.5±3.9	NS
Weight (Kg)	66.0±5.2	63.5±6.1	NS
BMI (Kg/m <sup>2</sup> )	23.1±2.3	22.7±1.8	NS
WC (cm)	83.1±7.9	84.6±6.1	NS
% BF	21.7±1.8	19.7±2.1	NS
<b>Nutrient intake</b>			
Protein (g/day)	40.3±9.1	39.8±6.9	NS
Energy(Kcal/day)	2080±122	2240±165	NS

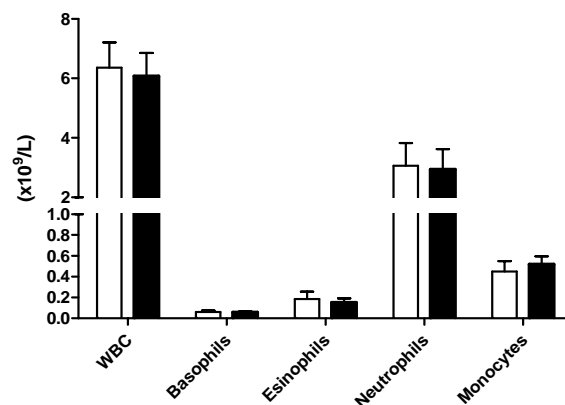
NS=non-significant by Student's t-test taking *p*≤0.05

### Difference in the number of platelets, WBC (and its subsets) between the elderly and young

Figure-1 shows that young had significantly more platelets than the elderly (551 vs 513 counts/L) (*p*<0.0001). Figure-2 shows a comparison between elderly and young regarding the number of WBC and its differential counts. As evident, between the elderly and young, there were non-significant differences in the mean number of WBC (6.07 x10<sup>9</sup>/L vs 6.35x10<sup>9</sup>/L), basophils (0.059 x10<sup>9</sup>/L vs 0.060 x10<sup>9</sup>/L), esinophils (0.152 x10<sup>9</sup>/L vs 0.186 x10<sup>9</sup>/L), and neutrophils (2.93 x10<sup>9</sup>/L vs 3.06 x10<sup>9</sup>/L) (*p*, all trends >0.05). In the elderly compared to young, there were significantly more monocytes (0.52 x10<sup>9</sup>/L vs 0.45 x10<sup>9</sup>/L) and lesser lymphocytes (2.41 x10<sup>9</sup>/L vs 2.60 x10<sup>9</sup>/L).



**Figure-1.** Platelets number in young (white bar) and old (black bar) women.



**Figure-2.** WBC and its subsets in young (white bar) and old (black bar) women.

### Relationship between age, platelets, WBC and its differential counts

The number of platelets decreased significantly with increasing age (*p* > 0.0001). The number of WBC and its subsets (basophils and neutrophils) decreased with increasing age. However, these trends were statistically non-significant (*p*, for all trends >0.05). Monocytes



significantly increased with increasing age ( $p=0.0096$ ), while lymphocytes decreased with age but only with a borderline statistical significance ( $p=0.051$ ).

## DISCUSSIONS

The main objective of the current study was to investigate the effects of aging on the number of platelets, WBC and its subsets. As reported in this study, young had significantly more platelets as compared to the elderly (Figure-1). Similarly, young had more WBC, eosinophils, neutrophils, and lymphocytes as compared to the elderly (Figure-2).

Aging has some effect on blood cells (Alam *et al.*, 2012a; Alam *et al.*, 2012d; Alam *et al.*, 2012e; Alam *et al.*, 2012f). The amount of fat in the marrow increases with age, which means there is less cell-producing marrow. While this decrease generally does not cause problems, it may when the body experiences an increased demand for blood cells: the marrow of an older person may be less able to meet those increased demands. Patients may present with anemia, low white blood cell or platelet counts, singly or in combination. The degree of low blood counts could be mild, moderate or even severe. It is quite common in the elderly.

In the present study, the number of platelets decreased significantly with increasing age ( $p>0.0001$ ). The number of WBC and some of its subsets (basophils and neutrophils) decreased with increasing age. However, these trends were statistically non-significant ( $p$ , for all trends  $>0.05$ ). Monocytes significantly increased with increasing age ( $p=0.0096$ ), while lymphocytes decreased with age but only with a borderline statistical significance ( $p=0.051$ ). Previous reports have suggested that aging may contribute to lymphocytes reduction. This reduction has been mainly attributed to a decreasing inducible lymphocyte proliferation and declining thymulin activity (Omran and Morley, 2000; Chandra, 2002; Walrand *et al.*, 2001; Kuzuya *et al.*, 2005; Izaks *et al.*, 2003). In female in particular, changes in hormonal levels have been attributed to cause changes in the number of WBC and its subsets (Khoram *et al.*, 2001).

Age has been identified as a variable influencing, for example, platelet count (Saxena *et al.*, 1987; Bain, 1996; Segal and Moliterno, 2006; Biino *et al.*, 2011). Bain *et al.* (1996) first showed that platelet count varied according to age, gender and ethnicity, findings which were subsequently confirmed by Segal and Moliterno, 2006. More recently, Biino *et al.*, (2011) showed, in a Sardinia geographic isolate population including subjects with a large age range that platelet count progressively decreased during aging, with a consequent increase of cases with thrombocytopenia and a decrease of cases with thrombocytosis in the elderly.

## CONCLUSIONS

We conclude that normal aging has a significant effect on the number of platelets, WBC and its fractional counts. Most of the cells decrease with increasing age. These changes may have important clinical implications which need further investigations.

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