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HAEMATOLOGICAL EFFECTS AND SAFETY BEHAVIOUR OF 20 kDa PEGINTERFERON ALPHA-2A (UNIPEG®) IN HEALTHY HUMAN SUBJECTS

*Ghousia. Saba[1], Javeid. Iqbal[1], Tasneem. Ahmad[2], Ayaz Ali Khan[1]

[1]Faculty of Pharmacy, Hamdard University, Karachi, Pakistan; [2]Pharma Professional Services, A-93, Ettawah Society, Scheme 33, Karachi 7540, Pakistan

***Corresponding author e-mail:** gsmush@yahoo.com

ABSTRACT

20 kDa pegylated interferon alpha-2a (UNIPEG®) manufactured through the pegylation of E.coli-derived interferon alpha-2a. The hematological effects of Unipeg® were studied after a single subcutaneous dose of 180 µg (protein content) in ten healthy human subjects. Vital signs, adverse events and lab tests (hematological and biochemical) were monitored for safety analysis. Clinical laboratory tests results before and after drug administration were processed for any statistically significant change by computing the ANOVA (F-value) and performing t-tests. Statistically significant decrease in neutrophils, absolute neutrophil count, total white blood cells count and platelets count were observed but most of these were not clinically significant. Transient leucopenia and leucopenia was reported for 10% and 20% subjects, respectively, transient thrombocytopenia in 10% subjects and an increase in ALT levels above normal ranges in 30% of subjects were observed while no serious adverse effect was reported. The hematological effects were found similar to those of reported in literature for unmodified IFN-alpha-2a and other pegylated IFN-alpha-2a products generally used in therapy.

Key words: PEG-IFN- alpha 2a, 20-kDa, Unipeg, Pegylated interferon alfa-2a, safety.

INTRODUCTION

The 20 kDa pegylated interferon alpha-2a (PEG(20kDa)-IFN-alpha-2a) is a covalent conjugate of the protein interferon alpha-2a (IFN-alpha-2a); produced by recombinant DNA technology in Escherichia coli, with a single stranded polyethylene glycol (PEG) molecule (20 kDa). [1] Interferon alpha-2a is a naturally occurring glycoprotein which belongs to the class of cytokines and mainly produced in the body by leukocytes. It plays an important role as immune stimulant or in the up gradation of immune system against viral infections and tumor cells.[2] It has antiviral, anti-proliferative and immunoregulatory properties. [3] This IFN-alpha-2a is susceptible for enzyme degradation and rapid clearance. Due to this property, IFN needs to be administered frequently; this leads to wide fluctuations in blood levels and interference in sustained therapeutic response. In addition, the varying IFN peak levels elicit more adverse effects. [4] These disadvantages limit its clinical usage. But, with the advent of pegylation in 1970, it was found that chemical modification of proteins can improve their therapeutic use; with added convenience of once-weekly dosing with no novel toxicities.[4] PEG optimizes the action of the IFNs by decreasing clearance rates, thereby allowing serum concentrations to remain constant over the dosing period and hence enhance efficacy. [5][6][7]

Polyethylene glycol (PEG) is a polymer consists of repeating ethylene oxide units and two terminal hydroxyl groups. Due to its production process PEG is available as a mixture of molecules with a variation of chain length, this phenomenon is termed polydispersity. These mixtures are usually classified by their average molecular weight, a value that may vary between several hundred to several thousand Daltons Both linear and branched PEG molecules are available.[8] The PEG used in study drug (UNIPEG; manufactured by Getz Pharma (Pvt) Ltd, Karachi,

Pakistan) has a limited polydispersity of 20,000±2,000. Pegylation of IFN-alpha-2a optimizes its pharmacological activity; such that the efficacy is enhanced, while adverse effects, antigenicity and immunogenicity are minimized and less frequent dosing is required; which enhanced the patients' compliance. [4][9]

Change in PEG chains (either linear or branched) attached to the protein molecule; results in pegylated interferons (IFN-alpha-2a) with different chemical properties, molecular weight, pharmacokinetic and pharmacodynamic properties.[4][8] The PEG-IFN-alpha-2a containing PEG chains of various chemical structures, size and molecular weight are commercially available in different countries of the world; e.g. 40kDa "Pegasys" (by Hoffmann-La Roch), 12kDa "PegIntron" (by Schering-Plough), 20 kDa, "Reiferon Retard®" (by Rhein Minapharma).[10][11][12] But, all these pegylated interferon therapies result in hematological cell suppression and causes cytopenia (thrombocytopenia, leucopenia, anemia, neutropenia). The aim of this study is to evaluate the hematological effects of locally manufactured PEG(20kDa)-IFN-alpha-2a in healthy human subjects.

MATERIAL AND METHOD

Investigational drug product: Each one ml ampoule of the Investigational drug UNIPEG® manufactured by Getz Pharma Pvt Ltd. Karachi, Pakistan contained PEG(20kDa)-IFN-alpha-2a 180µg protein. Besides the active ingredient the product also contained sodium chloride, acetic acid, sodium acetate, benzyl alcohol, tween 80, and water for injection as excipients.

Subjects: The study was conducted on ten healthy human volunteers with due approval of Independent Ethics Committee in compliance to the Declaration of Helsinkiand ICH-GCP guidelines. All volunteers gave their free written informed consent prior to participation. For participation in the study only those individuals were considered who had a BMI of 18-26 Kg/m2, age: 18-45 years, healthy with no history of chronic diseases, did not suffer in any acute illness in the previous 30 days, had no untoward symptoms or signs revealed through physical examination and laboratory tests, and were negative to HIV and hepatitis B and C virus. Subjects were not included if they had received the treatment with interferon (IFN) with in last one year or had donated blood in the previous 2 months. Individuals with low blood counts and hematology results outside the normal range were not included. Absolute Neutrophil Count (ANC) less than 1500/mm^3 and platelet count less than 50,000/ mm^3 were exclusion criteria.

Study Design: An open label, single dose study was conducted on ten healthy volunteers. 180mcg/ml of Unipeg® was injected in abdominal skin of the human volunteers. During the study, participants remained in the clinical facility for first 24 hours after the injection under strict medical supervision, then subjects were discharged and they visited the clinical facility at 36 hours then every 24 hours for physical check-up, vital signs and adverse event monitoring until 156 hours. Safety was monitored by complete physical and medical examination with clinical laboratory testing and scheduled vital signs surveillance. Clinical laboratory testing included hematological (hemoglobin, hematocrit, platelet, and total and differential leukocyte counts) and biochemical (transaminases) determinations as safety variables. Hemoglobin, ALT, Total WBC, Neutrophile % and absolute neutrophil count were conducted at screening and the values were taken as base line. On the fifth and 16^{th} day after drug administration the tests were repeated and the results were compared with the base line values. Paracetamol was given orally after observing the raise in body temperature after dosing and every 6 hours thereafter up to 24 hours or more if needed.

The Clinical Investigator closely monitored the subjects for adverse events (AE) and took all necessary actions in the best interest of subjects.

Data Analysis: Vital signs and Clinical lab tests results before drug administration and afterwards were processed for evaluation of any statistically significant change by computing the ANOVA (F-value) and performing t-tests. Simple statistics were employed in the computation, in summary the statistical formulas employed are:

Mean

$$\bar{x} \text{ or } \bar{y} = \sum \frac{x}{n} \text{ or } \sum \frac{y}{n}$$

SEM

$$SEM = \frac{\delta_{n-1}}{\sqrt{n}}$$

Variance ratio

$$F = \frac{\delta^2_{n-1}}{\delta^2_{n-1}}$$

In ANOVA F is determined by

$$F = \frac{MS_{treat}}{MS_{error}}$$

Percentage of volunteers suffered in different adverse events was also calculated.

RESULTS AND DISCUSSION

Laboratories Abnormalities or Hematological Effects: Interferon alpha 2a (IFN-alpha-2a) exerts anti-proliferative effect on many cell types. These properties are used for treatment of chronic myelo-porliferative and lympho-porliferative disease. But, this also results in hematological cell suppression or undesirable effects. IFN-alpha-2a therapy causes cytopenia (thrombocytopenia, leucopenia, anemia, neutropenia) and its main mechanism is seems to be bone marrow suppression especially for pleuripotent progenitor cell of all lineage which cannot be counteracted by the production of endogenous hematopoietic growth factor. IFN-alpha-2a therapy may also results in immune mediated hematological toxicity and capillary sequestration of platelets and WBC and seems to be the additional causes for thrombocytopenia and leucopenia. [13][14] Usually a normal complete blood count is recovered on discontinuation or dose modification of IFN-alpha-2a therapy. In this study the level of significance was determined for laboratory tests performed at screening, on day five and sixteen after single dosing; given in table 2.

Neutropenia: In the present study after single dose administration of Unipeg® (PEG (20kDa)-IFN-alpha-2a) in healthy volunteers, ANC remained above 1500/mm^3 and no neutropenia was identified (shown in figure 1). While, with PEG(40kDa)-IFN-alpha-2a in healthy volunteers; neutropenia was reported in 5% volunteers in a single study submitted in BLA 103964 by Hoffmann-La Roche. In this study, 180 mcg dose of PEG(40kDa)-IFN-alpha-2a was administered either subcutaneously or intravenously.[15] Marcellin et al. reported that neutropenia was the most common laboratory abnormality leading to dose modification during treatment with PEG(40kDa)-IFN-alpha-2a in patients. [16] Neutropenia after PEG(40kDa)-IFN-alpha-2a administration in patients has been reported from 17 to 47.5%. [17][18][19]

Decrease in Neutrophil Count: In present work it was observed that although single dose of PEG (20kDa)-IFN-alpha-2a did not cause neutropenia in any patient however, a decrease in neutrophil count from baseline values were identified in 100 % (10) volunteers but all these were within normal limits (i.e. 40-78%) with recovering trend towards their baseline values (shown in figure 2). In a single dose study of PEG(40kDa)-IFN-alpha-2a a decrease in neutrophil counts has also been reported in 50% of volunteers. In this study it was observed that thirty subjects had a total of 109 abnormalities, the majority which were decrease neutrophil counts (50%) and white blood cell count (30%). [20] Decrease in neutrophil count have also been reported in 17% volunteers for a PEG(40kDa)-IFN-alpha-2a study in healthy volunteers while evaluating its safety at 90, 180, and 270 µg subcutaneous dose.[15]

Thrombocytopenia: Thrombocytopenia mainly causes the dose modification of interferon alpha 2a therapy in patients. García-García et al identified thrombocytopenia as most frequent event in healthy subjects (i.e. in 56.3% volunteers; platelets counts less then 150X10^9 cells/L) after PEG(40kDa)-IFN-alpha-2a single dose injection. [21] In current study one case of transient thrombocytopenia and one case of very mild thrombocytopenis was observed. Taha et al studied the Reiferon Retard® in chronic hepatitis C (CHC) patient and reported that 12.15% patients suffered with thrombocytopenia during 24 to 48 weeks treatment period.[17] Thrombocytopenia has been observed in studies of PEG(40kDa)-IFN-alpha-2a in different conditions e.g. renal cell carcinomas, chronic hepatitis C, HIV and hepatitis B in different percentages ranging from 6% to 17.2%. [16][17][18][19][23][24][25][26] Marcellin et al. also reported thrombocytopenia and identified it as the major cause of dose modification for PEG(40kDa)-IFN-alpha-2a.[16] However, in this present study after single dose of PEG(20kDa)-IFN-alpha-2a in healthy patients only one (10%) case of transient thrombocytopenia was observed with recovering trend and two cases (20%) of very mild thrombocytopenia were reported. Transient decrease in platelets count have also been reported in 33% (4) volunteers at a dose of 180 µg of PEG(40kDa)-IFN-alpha-2a in healthy volunteers while evaluating its safety at 90, 180, and 270 µg subcutaneous dose.[15]

Serum glutamic-pyruvic transaminase /Alanine transaminase (SGPT/ALT): In our current study on day five, an increase from 5.5% to 108.69% in SGPT from baseline values was found in seven volunteers. But all these were within normal limits except for three volunteers (30%) in which SGPT raised above the normal value (above 41U/L) that is 48, 60 and 61 U/L. Out of these three; two subjects recovered on day sixteen of drug administration while one still had raised SGPT (64U/L). this indicates a transient elevation of ALT which returned to normal. García-García et al has also reported the increased transaminases (>100 UI) in 62.5% (n=10 out of 16) volunteers after single subcutaneous dose of PEG (40kDa)-IFN-alpha-2a. [25] Zeuzem et al. studied the PEG (40kDa)-IFN-alpha-2a plus ribavirin treatment in chronic hepatitis C patients with normal ALT levels compared to the patients with no treatment. He

concluded that transient elevations in ALT activity in treated and control patients during the study. No severe flares of ALT activity were associated with treatment, and the benefit-risk ratio was positive. [26]

Absolute neutrophil count (ANC): In the present study a decrease in absolute neutrophil count (ANC) was observed in 100% or all volunteers. It (ANC) dropped in a range of 24.42% to 75.98% on day five. But, ANC remained above 1500/mm^3. On day sixteen, recovering trend towards baseline value was observed for ANC in all volunteers. In seven volunteers, at follow-up; drop in ANC was 2.04% to 24.97% while it was 42.58%, 52.65% and 68.98% in three volunteers respectively. Although, the drop was marked but, no clinically significant neutropenia was reported. Varunok et al evaluated pharmacokinetics, user handling, and tolerability of PEG (40kDa)-IFN-alpha-2a delivered via a disposable auto injector device in 50 healthy volunteers; all subjects received one 180 µg dose. He found a decrease in neutrophil counts in 50% patient that definitely have been resulted in decrease of ANC. [20] Taha et al also observed neutropenia in 17.76% patients during treatment with Reiferon Retard® (PEG (20kDa)-IFN-alpha-2a) which indicates a decrease in absolute neutrophil count also. [17]

Leucopenia or decrease in total leucocytes count: In the current study with PEG (20kDa)-IFN-alpha-2a a decrease in leucocytes count was recorded for all volunteers (100%). On day five after drug administration WBC count drop from 27.4% to 60.97%. But, all these were within normal limits of WBC count (i.e. 4.0-11.0X10^9/L); except for three volunteers who had leucopenia with a drop of 33.33%, 47.69% and 51.61% respectively. The recovering trend towards baseline value of TLC was observed on day sixteen of drug administration; except for two volunteers (20%) who still had leucopenia (shown in figure 3). García-García et al also reported leucopenia 87.5% volunteers after a single dose of 180 mcg.[25] Decrease in WBC count have also been reported in 67% (n=8 out of 12) volunteers at a dose of 180 µg of PEG (40kDa)-IFN-alpha-2a in healthy volunteers while evaluating its safety at 90, 180, and 270 µg subcutaneous dose.[15]

Serious adverse events and death: In current study no serious adverse event or death has occurred. Similarly, no death and serious adverse event have been reported in any other single dose study of PEG (40kDa)-IFN-alpha-2a.[15][17][25]

CONCLUSION

PEG (20kDa)-IFN-alpha-2a (Unipeg®) administered subcutaneously as a single dose, shows hematological effects similar to other IFN-alpha-2a products. Although a decrease in neutrophil count, WBC, Absolute neutrophil count and platelets count was observed, none indicated neutropenia or clinically significant abnormality.

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Table 1: Demographic and baseline characteristics of subjects

Gender	**All Male**
Age (years)	25.2 ± 5.33 (20-32)
Height (Cm)	166(160-170)
Weight (kg)	59.60 ± 7.71 (60-74)
BMI (Kg/m2)	21.65 ± 2.71 (18.0-25.5)
Race	South Asians (Pakistani)
Mean Hb(gm)	14.45
Mean WBC	8X10^9/L
Mean Absolute Neutrophil Count	4500/mm^3
Mean Platelets Count	250X10^9/L

Table 2: Analysis of variance and p-value for clinical lab results

Parameter	**During the study (on fifth day of drug administration)**				**After the study on follow up (on day 16 of drug administration)**			
	F-value	p value	Sig at 1% level of significance	Sig at 5% level of significance	F-value	p- value	Sig at 1% level of significance	Sig at 5% level of significance
Hemoglobin (14-18g/dl)	0.6164	0.4426	Ns	Ns	1.5480	0.229	ns	ns
Total WBC 10^9 /l	21.718	0.00019	√	√	7.605222	0.0129	√	√
Neutrophils%	5.4706	0.0311	Ns	√	0.411673	0.529	Ns	Ns
Absolute neutrophils count	15.7624	0.00089	√	√	5.12642	0.036	Ns	√
Platelates 10^9 /l	9.477	0.0064	√	√	0.3008	0.590	Ns	Ns
ALT	2.8238	0.110	Ns	Ns	--	--	Ns	Ns
RBC	0.0592	0.8105	Ns	Ns	0.2733	0.607	Ns	Ns
PCV%	0.7848	0.387359	Ns	Ns	1.0463	0.319	Ns	Ns
MCV	0.2148	0.64858	Ns	Ns	0.1117	0.742	ns	Ns
Lymphocytes (20-45%)	9.4967	0.00643	√	√	1.013	0.327524	ns	Ns
Monocytes (1-10 %)	5.1984	0.03501	Ns	√	3.5095	0.077349	ns	Ns
Eosinophil (1-5%)	6.21547	0.02263	ns	√	1.9625	0.178251	ns	Ns

Ns: Not significant

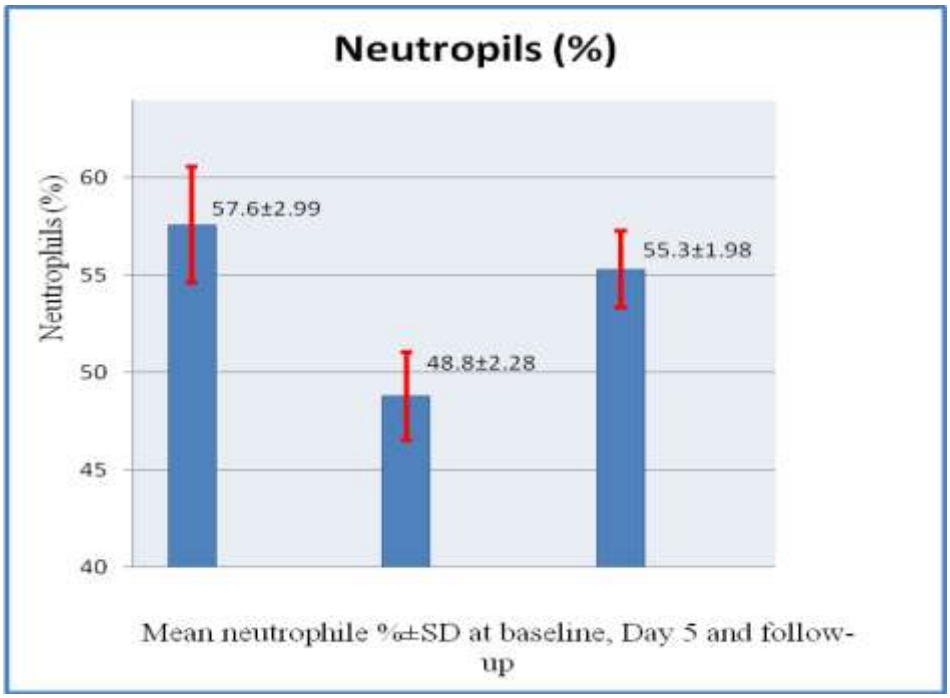

Figure 11: Mean neutrophils percentage observed in the study

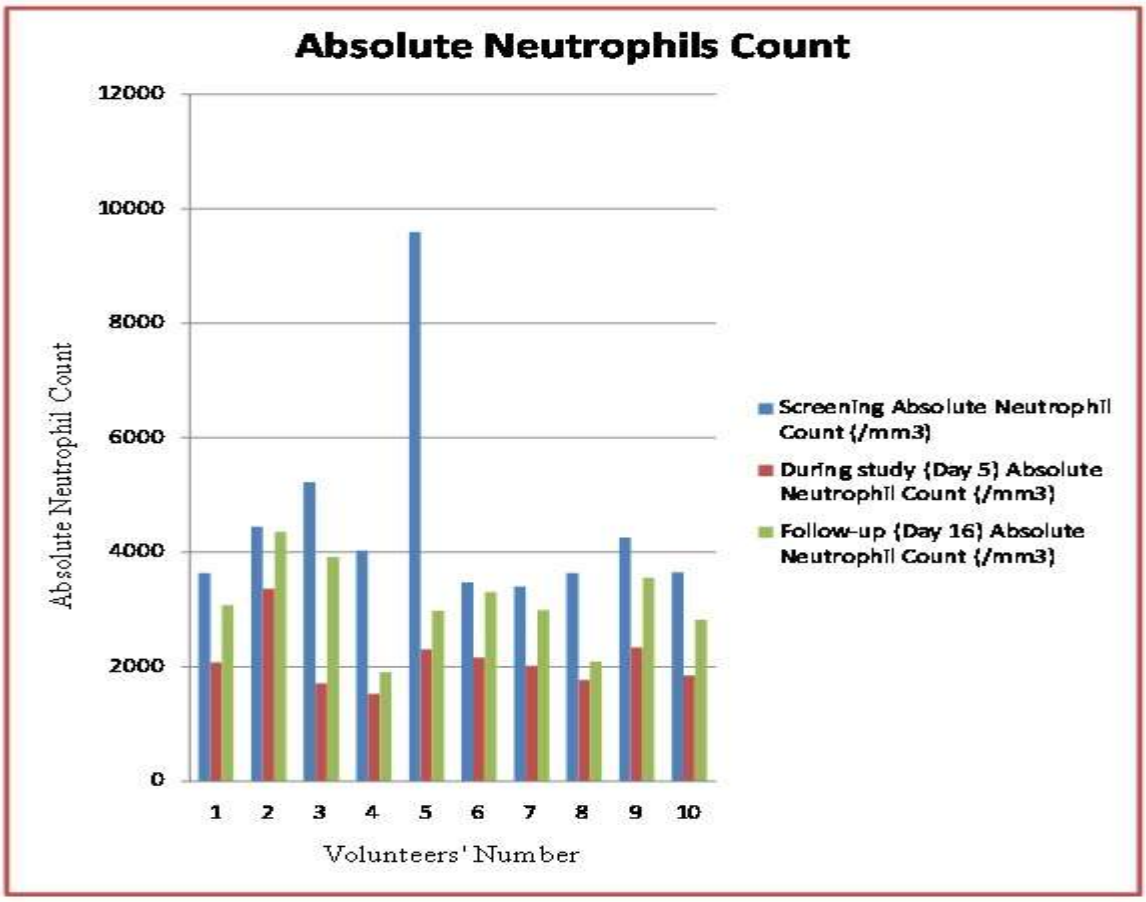

Figure 2: Change in absolute neutrophil count in all volunteers

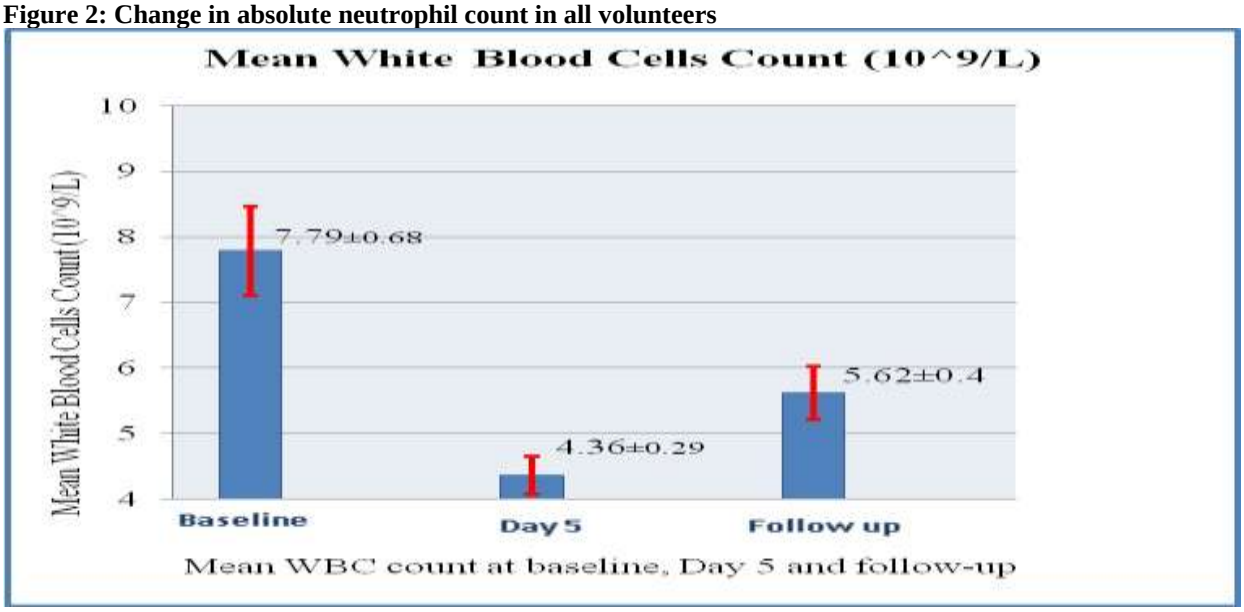

Figure 3: Mean WBC count observed in the study

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