Introduction

Haemoglobin is the iron holding and oxygen carrying protein found inside the red blood cells. This protein delivers many vital nutrients to human life [1]; furthermore, conveyances the toxic constituent carbon dioxide to the lungs for exhalation.

Humanoid haemoglobin is molded commencing two units of globin chains with a haem group. Seven diverse globin groups are produced in healthy subject; four are transitory developing hemoglobin stated as Haemoglobin Gower 1, Haemoglobin Gower 2, Haemoglobin Portland 1, and Haemoglobin Portland 2. Haemoglobin F is the main Hb of fetus lifecycle and includes the key fraction of Hb set up at delivery. Haemoglobin A is a key Hb that originates in grown-ups and kids. Haemoglobin A2 and Haemoglobin F are established in minor amounts in fully-grown (almost 2-3.3% and 0.2-1.0%, in turn). The grownup amounts of Hb A, A2, and F are typically achieved by 6-12 months [2,3]. It is a conjugated protein of molecular weight 64000 consisting of twosomes of polypeptide chains to each of which a haem is attached. The structure of the haemoglobin molecule may be viewed at four levels of organizational complexity. The basic arrangement of linked amino acids forming four polypeptide chains, each attached to a haem molecule is the primary structure. Each chain is arranged in a series of eight helical segments joined by short non-helical segments, and this is referred to as the secondary structure. The folding of each twisted chain into a specific three-dimensional configuration is the tertiary structure. The four folded chains fit closely together to form a compact tetrameric molecule known as the quaternary structure [4]. Studies have been done on hb level variations in males and females and it is found that women have 12% less hb level as compared to males of same race and origin due to Hb levels oxygen carrying capacity or need of oxygen in a body also differ in male and female [5]. Although, altitude was not considered in those studies but at high altitude oxygen level is low as compared to lower altitude and hb level also differ with the altitude [6].

Methods

Apparently healthy adult individuals with age ranging between 15 to 25 years (only males) were used as samples. Twenty-five (25) were the permanent resident of high altitude (7,500 feet from sea level) of Skardu valley and twenty-five individuals were from the low altitude (711 feet from sea level) belonging to Lahore. Total of these fifty individuals were the samples for this study. The study design was Comparative Observational and that did not involve any treatment. Estimation of hemoglobin concentration was done by Cyanmethemoglobin method [7,8] to compare the individuals HB from low altitude with high altitude.

When blood sample was diluted in a solution containing potassium cyanide and potassium ferricyanide (Drabkin's solution). It transforms haemoglobin (Hb) and methaemoglobin (Hi) to cyanmethaemoglobin (HiCN) which is a steady composite; this technique was used as standard for the study. The absorbance of the solution was measured in spectrophotometer at a wavelength of 540 nm which was associated with a standard solution of HiCN. Venous blood samples were collected in EDTA vials. 20 µl of blood from each sample was added to 5 ml of Drabkin's and well mixed by inverting the tube numerous times. It was then allowed to stand at room temperature for 3 – 5 minutes so that all Hb is transformed into HiCN. The absorbance was measured by using the spectrophotometer at 540 nm [8].

Absorbance of identified standard was also measured in the spectrophotometer with respectively each group of tests to reduce the chances of error. The estimation of Hb concentration was done by using the Cyanmethemoglobin method and the following formula was used for calculation: -

$$\text{Hb(g/L)} = \frac{\text{Absorbance of test x Concentration of standard}}{\text{Absorbance of Standard}}$$

Data collected was processed and analyzed using SPSS 17.0 (SPSS Inc. Chicago) for statistical analysis. The continuous variables were expressed in the form of Mean $\pm$ SD.

Results

25 individuals of high altitude gave these results of Hb (Mean= 15.1 and S.D =0.632). In the same way, 25 individuals from low altitude showed the following results of Hb (Mean= 14.5 and SD=0.633). There was a significant difference in Hb level at high altitude (7500 ft) and low altitude (711 ft) that is from the sea level. It gives the value for P= 0.004.

Sample	Hb level at higher altitude	Sample	Hb level at low altitude
1	13.89	1	13.89
2	16.06	2	13.45
3	13.54	3	13.98
4	14.54	4	14.69
5	14.96	5	14.19
6	14.93	6	14.75
7	14.89	7	15.19
8	14.93	8	14.78
9	14.95	9	14.64
10	15.99	10	13.99
11	15.91	11	13.98
12	14.97	12	14.67
13	14.98	13	15.78
14	14.96	14	14.98
15	14.99	15	14.56
16	14.98	16	14.78
17	14.96	17	14.88
18	14.99	18	14.01
19	14.95	19	16.09
20	15.95	20	14.76
21	13.85	21	13.68
22	14.95	22	13.48
23	14.99	23	14.89
24	14.88	24	14.29
25	16.09	25	14.99

Table 1: Hb level of the samples from lower and higher altitude

Discussion

Hemoglobin level in blood based on genders and origin of samples were studied previously but this study was conducted to find Comparison of hemoglobin level between high altitude people and low altitude people living in Pakistan. Individuals of high altitude have the mean value of Hb=15.1 and Standard Deviation=0.63. In the same way, mean and SD values were calculated for low altitude residents and the resulting mean value was 14.5 and SD was 0.633. There is a significant difference in Hb level at high altitude (7500 ft) and low altitude (711 ft from sea level), P = 0.004. This difference clearly indicates that altitude affects the level of hemoglobin in an individual. High value of Hb level was found in the people living at higher altitude as compared to the people of lower altitude. Significant difference of Hb values also determined the availability of oxygen in the habitat. Oxygen level is low at higher altitude so the people having high altitude habitat have high blood hemoglobin level to maintain the oxygen level and these changes are done by physiological and morphological adaptations acquired during the developmental period [9]. The purpose of this research is also to differentiate people of high altitude misleading diagnosed in lower altitude area hospitals as polycythemia rubra Vera because no proper data on hemoglobin levels of different altitude habitat were previously published in pakistan.

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Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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