Establishing Normal Ranges of Hematological Parameters From an Iranian Healthy Population: A Population-Based Cross-Sectional Study of Hospital Data

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Abstract- Measurement of hematological parameters and their reference ranges play an important role in the diagnosis and treatment of many infectious diseases and cancers. However, there are marked differences in the reference ranges between developing and developed countries. The aim of this study was to establish reference ranges of hematological parameters. This cross-sectional study was conducted in patients visiting Noor Eye Hospital who had no systemic diseases. In the lying position, blood samples were collected in two test tubes (Becton Dickinson Ltd, UK) using the Venoject method. EDTA-containing blood samples were used for complete blood count and differential leukocyte count using a cell counter (Nihdon Kohden Celltac E, Japan). Descriptive statistics, t-test, and ANOVA were used for data analysis. The data of 46,595 individuals were analyzed of whom 47.3% (n=22,042) were men. The mean (95% confidence interval) of white blood cells (WBC), red blood cells (RBC), platelet, and Hemoglobin (Hb) was 6.68 (6.66-6.69), 4.83 (4.83-4.84), 238.40 (237.87-238.93), and 14.29 (14.27-14.30), respectively. There was no difference in hematological parameters between male and female subjects. Except for the platelet count that was higher in individuals below 18 years than those 18-64 years and ≥65 years, other parameters had no relationship with age. Normal values of hematological parameters in the Iranian population are similar to the Middle East and African countries but below standard reference values. Except for the platelet count that decreased with age, there was no significant difference in hematologic and immunologic parameters between age and sex groups. Considering the difference between our results and standard reference values, we suggest that normal values be determined locally for each country.

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Keywords: Reference range; Hematological parameters; Iranian population; Adults; Ophthalmology

Introduction

Assessment of hematologic and immunologic factors is one of the most reliable para-clinical methods for diagnosis of a number of diseases (1,2). However, the level of these factors varies in different people as a result of between-subject (age, sex, genetic variation) and within-subject (pathological changes) differences or measurement error (3). Nonetheless, part of these differences is normal, and a range of changes is considered for hematological parameters (4).

Although there are different definitions for establishing a reference range of hematological parameters, a definition of mean±2 standard deviation is mostly accepted as the normal range (3,4). In many countries, the value obtained from the local population is compared against the reference provided by the WHO (3), but this comparison has two problems. First, standard reference values are based on an assessment of European and American populations which are different

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from the rest of the world in many aspects (1-5). Second, marked differences in hematological parameters have been reported across the world (6) which could be due to different factors like genetics, nutritional pattern, age, sex, and even geographic location (1,6-8). Therefore, it is not possible to determine a reference range for these parameters and generalize the results of these studies to developing countries (5). It is essential to establish local reference ranges for effective patient care (1), and comparison of the local reference range with standard reference values may increase the risk of unnecessary interventions or diagnosis failure (3). Therefore, many countries have made attempts to determine a reference range of their own (6,9-13). Studies have shown that African people have lower levels of hematological parameters than standard reference values (6,12). Moreover, some Asian (11) and Persian Gulf countries like Saudi Arabia (13) have reported lower levels of CD4 T-cell counts. Although the findings of these studies are different, they may improve the quality of health care services (3).

Considering the above, although there is a need for a comprehensive study in our country in this regard, few studies have been conducted to establish a reference range for hematological parameters, each with methodological errors. For example, some of them have only focused on a certain parameter (4,7) or age group (3) or their results cannot be generalized to the whole population (14). Therefore, we designed a study to cover the aforesaid and establish a reference range for all hematological parameters in different age groups with high generalizability.

Materials and Methods

Sampling and exclusion criteria

This cross-sectional study was conducted from 2011 to 2015. The data of all individuals visiting the Laboratory Department of Noor Eye Hospital were extracted from the hospital information system, and their hematological data were separated. These data can be considered a representative sample of the society because most patients visit the hospital for routine checkups or outpatient surgery on the visual system and have no systemic diseases affecting their hematological parameters. The exclusion criteria of the study were 1) being an immigrant or a foreign national, 2) HIV or AIDS positivity, 3) hepatitis B or C, 4) positive history of systemic diseases, 5) Cr>3, 6) any retinopathy or related diseases, 7) cancer, anemia, or any autoimmune disease like leukemia.

Blood collection

Blood samples were collected after 12 hours fasting. In the lying position, blood samples were collected in two test tubes (Becton Dickinson Ltd, UK), one containing EDTA and one without EDTA, using the Venoject method. To separate the serum, after incubation for 30 minutes, blood samples were centrifuged at 1000 g for 10 minutes, and the serum was immediately separated from the sediment. Biochemical tests were done using the Hitachi 902, Japan, and Pars Azmon kits (Licensed by Diagnostic System-Germany). The ELISA method was applied for immunological tests using Diaplus kits. EDTA-containing blood samples were used for complete blood count and differential leukocyte count using a cell counter (Nihdon Kohden Celltac E, Japan). Moreover, peripheral blood smears were prepared and stained with Giemsa for direct White blood cells (WBC) differential count using a microscope, and the results were compared with percentages provided by the cell counter. If no discrepancy was noted, the data of the cell counter as percentage and number per microliter were used in the study. If there was a marked discrepancy between the results, the sample was excluded from the study. All samples were analyzed within 12 hours (15). Informed consent was obtained from all participants, and their permission was sought to publish their data anonymously.

Statistical analysis

Before the final analysis, the data with more than three standard deviations from the mean were considered as outliers and removed from the analysis. Descriptive statistics including mean, 95% confidence interval, median, and inter-quarter range (IQR) were used to show the status of hematological parameters. Percentiles and indexes of skewness and kurtosis were used to evaluate the distribution of these parameters. T-test and ANOVA were applied to evaluate the effect of sex and age (below 18 years, 18-64 years, above 64 years). If the results of ANOVA were significant, the Tukey post hoc test was used for pairwise comparison. In this study, mean±2SD was considered the normal range. SPSS 16 (SPSS Inc., Chicago, IL, USA) was used for analysis and *P* less than 0.05 were considered significant.

Results

The data of 47,414 individuals were collected. After

applying the exclusion criteria, the data of 46,595 subjects were included in the study. In some cases, all the parameters were not available; these cases were considered as missing and were not included in the final analysis. Considering the low percentage of missing cases, there were no concerns about bias (less than 5% for all variables).

In this study, 22,042 subjects (47.3%) were men and 24,553 (52.7%) were women. The mean age of the participants in the age group 0-18 years, 18-65 years, and above 65 years was 7.99±5.53, 53.29±11.91 and 73.85±5.83 years, respectively. Table 1 shows the mean (95% CI), median±IQR, 5, 25, 50, 75, and 95 percentiles, and amount of skewness and kurtosis for each hematological parameter. According to results of Table 1, the mean (95% CI) WBC, red blood cells (RBC), platelet, and Hemoglobin (Hb) was 6.68 (6.66-6.69), 4.83 (4.83-4.84), 238.40 (237.87-238.93) and 14.29 (14.27-14.30), respectively.

Table 2 shows the effect of age and sex on hematological parameters.

Table 1. Mean (95% confidence interval), Median, Percentile and Normality parameter for the Hematological parameter in the study

Parameter*	Mean (95% CI)	Median±IQR#	Percentile					Normality parameter	
			5	25	50	75	95		
RBC* (10^6/ µL)	4.83 (4.83 - 4.84)	4.80 ± 0.67	4	4.50	4.80	5.17	5.74	0.20	0.14
Hemoglobin (g/dl)	14.29 (14.27 - 14.30)	14.2 ± 2.10	11.70	13.20	14.20	15.30	16.90	0.03	-0.05
HCT (%)	42.50 (42.46 - 42.54)	42.5 ± 5.70	35.50	39.70	42.50	45.40	49.40	-0.01	-0.05
MCV (26)	88.83 (88.79 - 88.88)	89 ± 6.10	80.60	85.90	89	92	96.30	-0.21	0.36
MCH (26)	29.85 (29.83 - 29.87)	30 ± 2.4	26.20	28.80	30	31.20	32.80	-0.73	1.62
MCHC (g/dl)	33.62 (33.61 - 33.63)	33.70 ± 1.40	31.70	33.00	30.70	34.40	35.30	-0.36	0.32
Platelet *(10^3/µL)	238.40 (237.87 - 238.93)	234 ± 77	153	198	234	275	341	0.34	-0.04
WBC * (10^3/µL)	6.68 (6.66 - 6.69)	6.51 ± 2.04	4.40	5.60	6.51	7.64	9.51	0.42	-0.01
Neutrophil (count)	3.81 (3.80 - 3.83)	3.66 ± 1.49	2.22	2.99	3.66	4.48	5.95	0.74	0.72
Lymphocyte (count)	2.27 (2.27 - 2.28)	2.20 ± 0.89	1.29	1.79	2.20	2.67	3.52	0.77	1.21
Monocyte(count)	0.39 (0.38 - 0.39)	0.37 ± 0.34	0.10	0.20	0.37	0.54	0.77	0.57	-0.23
Eosinophil (count)	0.20 (0.19 - 0.20)	0.16 ± 0.14	0.06	0.11	0.16	0.25	0.45	1.67	3.73

* RBC: red blood cells; HCT: Hematocrit; MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: White blood cells.
# IQR: inter-quarter range

Table 2. Effect of sex and age on the value of the hematological parameter in the study population

Parameter*	Sex Effect		*P*	Age Effect			*P*
	Male	Female		≤18	19-64	≥65	
RBC* (10^6/ µL)	4.83 ± 0.51	4.83 ± 0.52	0.27	4.83 ± 0.49	4.83 ± 0.51	4.83 ± 0.52	0.47
Hemoglobin (g/dl)	14.29 ± 1.56	14.28 ± 1.58	0.52	14.29 ± 1.57	14.28 ± 1.56	14.29 ± 1.58	0.72
HCT (%)	42.52 ± 4.17	42.49 ± 4.22	0.43	42.48 ± 4.13	42.48 ± 4.18	42.52 ± 4.22	0.69
MCV (26)	88.85 ± 4.78	88.82 ± 4.75	0.49	88.94 ± 4.70	88.80 ± 4.77	88.85 ± 4.77	0.37
MCH (26)	29.85 ± 2.07	29.85 ± 2.05	0.77	29.87 ± 2.02	29.85 ± 2.06	29.86 ± 2.05	0.83
MCHC (g/dl)	33.62 ± 1.09	33.63 ± 1.09	0.63	33.61 ± 1.11	33.63 ± 1.09	33.62 ± 1.09	0.73
Platelet *(10^3/µL)	238.11 ± 57.17	238.66 ± 57.03	0.30	243.62 ± 60.09	239.05 ± 57.04	237.53 ± 56.96	<0.001#
WBC * (10^3/µL)	6.68 ± 1.53	6.68 ± 1.53	0.83	6.75 ± 1.55	6.68 ± 1.53	6.67 ± 1.54	0.25
Neutrophil (count)	3.82 ± 1.14	3.81 ± 1.15	0.83	3.86 ± 1.16	3.81 ± 1.15	3.81 ± 1.14	0.40
Lymphocyte (count)	2.27 ± 0.69	2.27 ± 0.69	0.76	2.29 ± 0.67	2.27 ± 0.69	2.27 ± 0.69	0.54
Monocyte(count)	0.38 ± 0.21	0.39 ± 0.21	0.49	0.39 ± 0.21	0.39 ± 0.21	0.38 ± 0.21	0.26
Eosinophil (count)	0.20 ± 0.12	0.20 ± 0.12	0.86	0.19 ± 0.12	0.20 ± 0.12	0.20 ± 0.12	0.65

* RBC: red blood cells; HCT: Hematocrit; MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: White blood cells.
# significance at 0.05

According to Table 2, sex had no impact on hematological parameters. Moreover, except for the platelet count (P<0.001), age also had no effect on hematological values. The Tukey post hoc test was used for a more accurate assessment of the effect of age on the platelet count. The results showed that a higher platelet count in individuals <18 years than those aged 18-64 years (P=0.015) and above 65 years (P=0.001) (Figure 1). Subjects aged 18-64 years had more platelets than those above the age of 65 years (P=0.015).

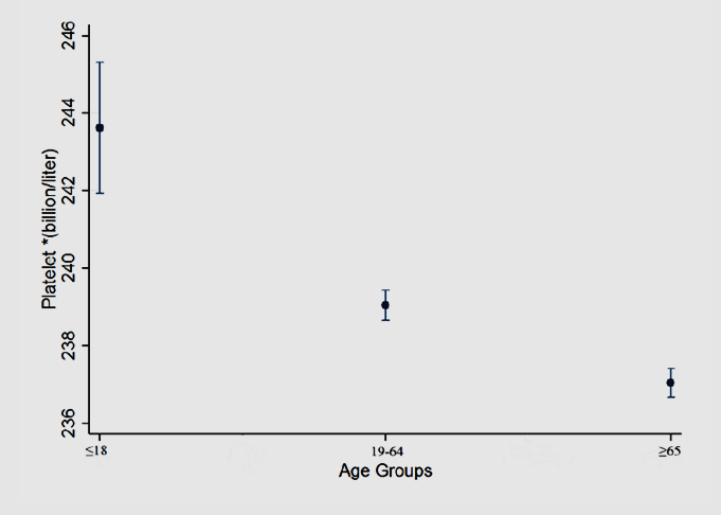

Figure 1. Error bar of platelet count (10^9/liter) in the study population. Each line donated standard error of the mean

Discussion

This was the first Iranian study with the aim of establishing normal values of hematological parameters in the Iranian population. It should be noted that the definition of normal values for immunohematological parameters depends on the context and clinical, epidemiological, and statistical factors (3). However, a definition of mean±2 SD is widely accepted and used based on statistical and epidemiological concepts (3,4). We also used this definition in our study.

Many studies have investigated the normal values of hematological parameters in different populations in the recent five decades (1,5-8,11,12,15-20). Comparison of the results of these studies shows that in addition to differences in the values of hematological parameters between populations, the recommended standard values (21) are higher than the values obtained in African, Middle East, and Asian countries (12), mainly due to environmental factors as well as altitude (18).

According to the results of our study, the normal values of many hematological parameters in the Iranian population are different from standard reference values. The standard reference values of Hb is 15.5 (95% CI: 14,2-18.1) (21), but the Hb level was borderline in our study (14.29, 95% CI: 14.27-14.30). The level of Hb in our study was similar to reports from Uganda (15), Indonesia (22), and Africa (16), but higher than a report from Kenya (5). It seems the level of Hb in Iran and other developing countries is lower than standard reference values (21), which may be due to iron deficiency and other nutritional problems leading to the high prevalence of anemia in these regions (23).

Although we noted no difference in the Hb level between age and sex groups, some studies have shown that the female sex has a lower Hb level than the male sex (6,15,16). It seems that an important reason for this difference is menstruation (17). However, it should be noted that the prevalence of iron deficiency is higher in women, which may be another reason for this finding (23). However, hormonal and physiological differences between men and women may also be involved.

According to our findings, the RBC count in the Iranian population, regardless of age and sex, is lower than standard reference values (21) but is similar to some African countries (8,15,20) which may be due to the high prevalence of malaria and other hematological diseases like thalassemia in these countries (10).

In our study, mean cell volume (MCV) was similar to Saudi adolescents below 18 years (18) but below standard reference values (21). We found no significant difference in RBC, MCV, mean cell hemoglobin

(MCH), mean corpuscular hemoglobin concentration (MCHC), and Hematocrit (HCT) parameters between men and women. Some studies have reported higher aforementioned parameters in men for reasons such as hormonal differences and menstrual loss (1,20). Moreover, we found no differences in RBC, MCV, MCH, MCHC, and HCT parameters between different age groups while some other studies have shown that RBC and HCT are higher in older people as a result of higher androgen levels (1). This inconsistency may result from differences in ethnicity, race, or sampling method; however, more studies are warranted. In our study, the value of RBC and RBC subsets was similar to other studies in Iran (7) and some African countries like Uganda, (15) Ethiopia (20) and Sri Lanka (24), but below standard reference values (21). The reason for this difference is not clear but nutritional, environmental, and genetic factors may be involved (15). On the other hand, we found no difference in WBC between age and sex groups which is consistent with other studies (20,24).

The platelet count in our study was 238000 per microliter, which was consistent with the results of similar domestic studies (3,14) and studies conducted in African countries like Kenya (5), Central African Republic (6), and Uganda (15), but below values reported from European developed countries like Italy (16). This difference may result from genetic, environmental, or unknown factors (15,25). Many clinical laboratories consider $150\text{-}400*10^9$ platelets/L as the normal range. Although the platelet count was in this range in our study ($237.87\text{-}238.93*10^9$), it had a shift towards the lower limit of normal, indicating the importance of establishing local reference ranges.

We found no difference in the platelet count between men and women, which is consistent with the results of other studies (6). However, some studies have shown a higher platelet count in women (3,17) which may be due to menstruation in women (17). Our results also showed a significant decrease in the platelet count with age as individuals >65 years had the lowest platelet count than those aged 18-65 years and below 18 years. This finding is also reported in other studies (17). Although the reason for this finding is not clear, some authors have mentioned decreased thrombopoietin level (26) or decreased hematopoietic stem cell reserve with aging (17) as the reason. Further studies are required to elucidate the main reasons.

A large sample size, evaluation of all Complete Blood Count (CBC) factors and including all age ranges were the strong points of this study. Since most people visit Noor Eye Clinic for routine checkups or outpatient ocular operations, and the majority of ocular diseases have no effects on hematological parameters, the use of this dataset provided an opportunity to investigate a representative sample of the population. On the other hand, all study participants received hormonal and liver tests among other tests and therefore it was possible to detect and exclude cases with diseases affecting blood parameters, a point that is not usually possible in population-based studies (15). It seems that evaluation of the possible effect of environmental factors as well as nutritional and lifestyle habits on hematological parameters reported in several studies (15,23,25) can add to our existing knowledge of the determinants of hematological parameters which should be considered in designing future studies.

In conclusion, hematological parameters in the Iranian population are similar to the Middle East and African countries but different from standard reference values. Except for the platelet count that decreased with age, we found no significant difference in hematologic and immunologic parameters between age and sex groups.

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