FACTORS INFLUENCING LEUCOCYTE COUNTS OF PERIPHERAL BLOOD

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SYNOPSIS

Two hundred and ninety six subjects of both sexes comprising 68 Europeans and 228 Indians, resident in India, were studied with respect to factors which may influence the total and differential leucocyte counts. No consistent variation of the total and differential leucocyte counts was observed between the Europeans and Indians in the present study. However, male South Indian students had a significantly higher total leucocyte count than the male North Indian students ($p<0.01$). A significant decline of the number of monocytes and eosinophils was observed with advancing age among Indians ($p<0.01$). The packed cell volume was found to be significantly correlated with the monocyte ($p<0.005$) and eosinophil counts ($p<0.05$).

There was a tendency towards increased neutrophil counts in the winter, although this finding was not consistent in all groups studied. Among Europeans there was a tendency towards a decrease of neutrophil counts with the duration of stay in India. The total and differential leucocyte counts were not correlated with socio-economic status, diet, physique, blood groups (ABO and Rh), haemoglobin concentration, red cell indices, erythrocyte sedimentation rate and serum protein fractions.

INTRODUCTION

The total leucocyte count of peripheral blood has been reported to be lower in the Negroes in Africa, USA and United Kingdom compared to Caucasians. (1, 2, 3, 4, 5, 6) This decreased neutrophil count is called Neutropenia of African Origin, because it has not been found in Asian and European residents in Africa. In these cases neutropenia may or may not be associated with eosinophilia or lymphocytosis (2, 3).

British military personnel of the Royal Air Force working in the tropics were found to have a lowered leucocyte count which was predominantly due to a low neutrophil count (7). Parasitic infestation has not been found to be related to neutrophil count in African subjects (2, 4).

Factors which have been found to influence the total and differential leucocyte counts include race (1, 2, 5, 6, 8), age (9, 10, 11, 12, 13, 14), diet (15), environment (3, 7, 15), circadian rhythm

(16, 17), light cycle (18), radiation (17, 19), physique (20) and physical and emotional stress (21).

The aim of the present investigation was to study the relationship between some genetic and environmental factors and the total and differential leucocyte counts in Indians and Europeans resident in India.

MATERIALS AND METHODS

Two hundred and ninety six staff members and students of the Christian Medical College, Government College and Health Visitors School, Ludhiana, Punjab, who volunteered to participate were the subjects of this study. The age range was 17–55 years and all individuals were apparently healthy as judged by clinical and laboratory investigations. The sample was comprised of 68 Europeans (31 male and 37 female) and 228 Indians (110 male and 118 female) with different ethnic back-grounds, dietary habits and socio-economic status. Blood samples from 48 Indians and 26 Europeans were collected both in summer (July, August) and winter (January) to examine the seasonal variation of leucocyte counts. The summer and winter values of the counts were averaged in calculations involving relations with other variables. Height, nude weight of the subjects were recorded in the morning. The surface area was determined from the standard nomogram. Ponderal Index was calculated by the formula $H/\sqrt[3]{W}$ (22). Oral temperature, pulse and respiratory rates and blood pressure were recorded under resting condition before collection of blood. Ambient temperature, barometric pressure and relative humidity were measured each morning for the entire period of the experiment.

Fasting blood samples were collected by venepuncture in the morning after a period of rest to avoid the effect of stress. Blood samples of female subjects were collected at the midmenstrual cycle. The total and differential leucocyte counts, haemoglobin content, packed cell volume, erythrocyte sedimentation rate and blood groups (ABO & Rh) were determined by standard techniques. The total leucocyte count was estimated from the average of three readings and differential leucocyte count was calculated from 200 white cells (23). The methodology for other parameters was essentially the same as described earlier (24). The counts of leucocytes were expressed in 10^3 per mm^3 of blood (Mean ± S.D.). The significance of differences between groups was calculated by student's "t" test and presented as P values. The coefficients of correlations between five leucocyte counts (total leucocyte, neutrophil, lymphocyte, monocyte and eosinophil) and a number of variables (age, height, weight, surface area, ponderal index, haemoglobin concentration, packed cell volume, red cell indices, erythrocyte sedimentation rate, total protein, albumin, globulin, and fractions of globulins) were calculated by stepwise multiple regression analysis and Spearman's r correlations only on the data of Indian subjects excluding Dravidians due to their deviating results.

RESULTS

Ethnic group: Three ethnic groups were studied for the leucocyte counts (i) 68 European Officers resident in India for 1 to 10 years; (ii) Indians comprised of 53 officers from different parts of India and 147 medical students from northern India (iii) 28 South Indian medical students (Table 1). The mean total leucocyte count in the Europeans was 5.41 ± 1.19 x 10^3 per mm^3 of blood. In the Indian officers of comparable age and sex the mean total leucocyte count was 5.94 ± 1.25 x 10^3 per mm^3 of blood. North Indian students had a mean total leucocyte count of 6.04 ± 1.49 x 10^3. The Indian officers had a higher total leucocyte count than the Europeans, but the difference was not significant in either sex. South Indian male students had a significantly higher total leucocyte count than the male students from Northern India ($p<0.01$) mostly due to higher count of neutrophils, but the trend was reversed in the case of females. There was no consistent variations in differential leucocyte counts among the ethnic groups.

Age: The total and differential leucocyte counts of the different age-groups of Indian subjects are presented in Table 2. The South Indians have been excluded due to their unusual counts. The results of stepwise multiple regression and Spearman's r correlation of the total and differential leucocyte counts are shown in Table 7. There appears to be a gradual decrease in the number of all the leucocytes with advancing age, but the decrease is not statistically significant. However, the results of multiple regression and Spearman's r test show a significant decrease in the number of monocytes ($p<0.01$) and eosinophils ($p<0.05$) with advancing age.

Sex: It appears that women in general had a lower count of all the white cells (Table 2). But the difference is not statistically significant in any of the age groups excepting for the age group 21–30 years for the total leucocyte counts ($p<0.05$).

Socio-economic status: The subjects of the lower socio-economic class were a group of thirty seven female students attending Health Visitor's Training School and they were on the Government of India's stipendary help for being from poor homes. The group of higher socio-economic class was selected at random from among the female medical students of comparable age. Both the groups were comprised of North Indians only. The classification of socio-economic status was based on the patient's income and family size (Table 3). The group of higher socio-economic status showed a higher total leucocyte count (6.18 ± 1.44 x 10^3) compared to those from lower socio-economic status (5.68 ± 1.00 x 10^3). The difference was not statistically significant ($p>0.05$). The differential leucocyte count was similar in both the groups excepting a significantly higher count of monocytes in the higher socio-economic group ($p<0.01$).

Season: In 48 Indian subjects (30 males and 18 females) and 26 Europeans (13 males and 13 females), the total and differential leucocyte counts were determined both at the heights of summer and winter (Tabel 4). Total leucocyte count in each of the groups was higher in winter than that in summer mainly due to an increase in the number of neutrophils. But the diffe-

Table 1

Total and differential leucocyte counts in different Ethnic groups (Mean ± S.D.) in 10^3 per mm^3.

Ethnic groups	Status	Sex	No	Total Leucocytes	Neutrophils	Lymphocytes	Monocytes	Eosinophils
European	Officers	M	31	5.45 ± 1.28	2.90 ± 0.83	2.04 ± 0.44	0.26 ± 0.08	0.21 ± 0.12
		F	37	5.31 ± 1.13	2.96 ± 0.83	1.96 ± 0.49	0.25 ± 0.08	0.18 ± 0.08
		Both	68	5.41 ± 1.19	2.94 ± 0.82	1.99 ± 0.46	0.25 ± 0.09	0.19 ± 0.10
Indian	Officers	M	32	6.16 ± 1.23	3.13 ± 0.76	2.35 ± 0.64	0.27 ± 0.09	0.20 ± 0.14
		F	21	5.61 ± 1.23	2.92 ± 0.70	2.14 ± 0.55	0.24 ± 0.12	0.26 ± 0.16
		Both	53	5.94 ± 1.25	3.15 ± 0.75	2.27 ± 0.26	0.26 ± 0.10	0.22 ± 0.15
North Indian	Students	M	61	6.09 ± 1.74	3.21 ± 1.20	2.21 ± 0.66	0.31 ± 0.17	0.38 ± 0.30
		F	86	6.01 ± 1.35	3.29 ± 0.96	2.15 ± 0.51	0.28 ± 0.12	0.28 ± 0.21
		Both	147	6.04 ± 1.49	3.27 ± 1.05	2.18 ± 0.68	0.29 ± 0.14	0.32 ± 0.22
South Indian	Students	M	17	7.51 ± 1.57	3.86 ± 0.86	2.64 ± 0.59	0.31 ± 0.16	0.45 ± 0.30
		F	11	5.19 ± 1.03	2.67 ± 0.69	2.00 ± 0.39	0.31 ± 0.12	0.26 ± 0.14
		Both	28	6.69 ± 1.77	3.39 ± 0.99	2.39 ± 0.62	0.31 ± 0.15	0.38 ± 0.28

Table 2

Total and differential leucocyte counts in different age groups of Indians (Mean ± S.D.) in 10^3 per mm^3 (n = 201)

Age groups (yrs)	Sex	No	Total Leucocytes	Neutrophils	Lymphocytes	Monocytes	Eosinophils
17 - 20	M	34	6.18 ± 1.65	3.23 ± 1.13	2.23 ± 0.65	0.36 ± 0.13	0.37 ± 0.25
	F	59	6.12 ± 1.31	3.33 ± 0.94	2.18 ± 0.43	0.30 ± 0.13	0.29 ± 0.21
	Both	93	6.14 ± 1.43	3.29 ± 1.01	2.20 ± 0.52	0.33 ± 0.14	0.32 ± 0.23
21 - 30	M	43	6.10 ± 1.38	3.17 ± 0.76	2.26 ± 0.58	0.27 ± 0.15	0.33 ± 0.21
	F	36	5.35 ± 1.07	2.80 ± 0.70	2.04 ± 0.40	0.23 ± 0.12	0.28 ± 0.24
	Both	79	5.76 ± 1.29	3.00 ± 0.75	2.16 ± 0.51	0.25 ± 0.14	0.31 ± 0.23
31 - 40	M	11	5.97 ± 1.46	3.30 ± 1.09	2.20 ± 0.62	0.26 ± 0.08	0.17 ± 0.10
	F	9	5.53 ± 1.40	2.99 ± 0.90	2.06 ± 0.70	0.21 ± 0.07	0.23 ± 0.15
	Both	20	5.77 ± 1.41	3.16 ± 0.99	2.14 ± 0.64	0.24 ± 0.08	0.20 ± 0.13
41 - 55	M	8	5.93 ± 0.78	3.02 ± 0.28	2.49 ± 0.57	0.22 ± 0.06	0.15 ± 0.15
	F	1	7.41 ± 0.00	3.45 ± 0.00	3.11 ± 0.00	0.22 ± 0.00	0.60 ± 0.00
	Both	9	6.11 ± 0.90	3.07 ± 0.30	2.56 ± 0.57	0.22 ± 0.06	0.21 ± 0.17

Table 3

Total and differential leucocyte counts in persons coming from different socio-economic class (only females) (Mean ± S.D.) in 10^3 per mm^3

Socio-economic class	No	Total Leucocytes	Neutrophils	Lymphocytes	Monocytes	Eosinophils
Higher*	37	6.18 ± 1.44	3.29 ± 1.02	2.20 ± 0.43	0.36 ± 0.12	0.31 ± 0.22
Lower**	37	5.68 ± 1.00	3.09 ± 0.71	2.11 ± 0.43	0.20 ± 0.09	0.25 ± 0.20

* Female medical students:- Mess bill = Rs. 65.00 p.m. (Rs. 7.5 = 1U.S.$). Parents income more than Rs. 500.00p.m. with average family of five.

** Female health visitors:- Mess bill = Rs. 35.00 p.m. Parents income less than Rs. 200.00 p.m. with average family of six.

Table 4

Total and differential leucocyte counts in summer and winter (Mean ± S.D.) in 10^3 per mm^3

Ethnic groups	Season	Sex	No	Total Leucocytes	Neutrophils	Lymphocytes	Monocytes	Eosinophils
Indians	Summer	M	30	5.96 ± 1.62	3.03 ± 1.00	2.35 ± 0.80	0.27 ± 0.08	0.32 ± 0.16
	Winter	M	30	6.73 ± 1.73	3.69 ± 1.24	2.47 ± 0.50	0.29 ± 0.15	0.28 ± 0.22
	Summer	F	18	5.40 ± 0.95	2.82 ± 0.69	1.89 ± 0.58	0.28 ± 0.11	0.25 ± 0.13
	Winter	F	18	6.22 ± 1.39	3.57 ± 0.78	2.24 ± 0.47	0.34 ± 0.13	0.23 ± 0.09
Europeans	Summer	M	13	5.10 ± 0.94	2.63 ± 0.59	1.98 ± 0.31	0.24 ± 0.06	0.21 ± 0.14
	Winter	M	13	5.89 ± 1.43	3.20 ± 0.95	2.16 ± 0.50	0.29 ± 0.10	0.18 ± 0.05
	Summer	F	13	5.11 ± 0.88	2.75 ± 0.44	1.89 ± 0.52	0.26 ± 0.08	0.18 ± 0.06
	Winter	F	13	5.42 ± 1.46	3.11 ± 1.06	1.90 ± 0.58	0.25 ± 0.10	0.15 ± 0.08

Summer:- Average ambient temperature - 33°C
Average barometric pressure - 727 mm Hg
Average humidity - 75%

Winter:- Average ambient temperature - 11°C
Average barometric pressure - 734 mm Hg
Average humidity - 57%

Table 5

Total and differential leucocyte counts in Europeans according to duration of stay in India (Mean ± S.D.) in 10^3 per mm^3

Duration of stay in India	Season	Sex	No	Total Leucocytes	Neutrophils	Lymphocytes	Monocytes	Eosinophils
Less than 5 years	Summer	M	9	5.19 ± 1.00	2.78 ± 0.60	1.97 ± 0.34	0.23 ± 0.06	0.17 ± 0.09
		F	7	5.29 ± 1.20	2.66 ± 0.63	2.10 ± 0.56	0.28 ± 0.12	0.20 ± 0.11
		Both	16	5.24 ± 1.05	2.73 ± 0.60	2.03 ± 0.44	0.28 ± 0.09	0.18 ± 0.10
	Winter	M	9	6.46 ± 1.56	3.57 ± 1.01	2.29 ± 0.55	0.34 ± 0.09	0.22 ± 0.11
		F	10	5.91 ± 0.97	3.34 ± 0.60	2.11 ± 0.58	0.23 ± 0.08	0.18 ± 0.12
		Both	19	6.17 ± 1.28	3.45 ± 0.80	2.20 ± 0.56	0.28 ± 0.10	0.20 ± 0.11
More than 5 years	Summer	M	6	4.61 ± 0.80	2.27 ± 0.36	1.81 ± 0.39	0.23 ± 0.06	0.24 ± 0.13
		F	12	5.00 ± 0.74	2.69 ± 0.45	1.85 ± 0.45	0.25 ± 0.04	0.18 ± 0.06
		Both	18	4.87 ± 0.76	2.55 ± 0.46	1.83 ± 0.42	0.24 ± 0.05	0.19 ± 0.11
	Winter	M	5	5.12 ± 0.41	2.69 ± 0.50	1.98 ± 0.27	0.21 ± 0.05	0.24 ± 0.13
		F	10	5.48 ± 1.51	3.25 ± 1.27	1.79 ± 0.28	0.24 ± 0.11	0.18 ± 0.07
		Both	15	5.36 ± 1.25	3.06 ± 0.99	1.85 ± 0.28	0.23 ± 0.09	0.20 ± 0.09

Table 6

Total and differential leucocyte counts in persons of different dietary habits (Mean ± S.D.) in 10^3 per mm^3

Sex	No	Total Leucocytes	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Average Daily Nutrient Intake(27) Protein (gm)	Fat (gm)	Carbohydrate (gm)	K-calorie
				Vegetarian						
M	12	6.14 ± 1.54	2.91 ± 0.78	2.35 ± 0.57	0.32 ± 0.15	0.53 ± 0.47	77.7	64.5	253.8	1905
F	29	5.99 ± 1.46	3.27 ± 1.13	2.15 ± 0.48	0.26 ± 0.16	0.29 ± 0.20	51.0	36.0	155.0	1154
Both	41	6.03 ± 1.48	3.16 ± 1.03	2.21 ± 0.51	0.28 ± 0.16	0.36 ± 0.34				
				Non-Vegetarian						
M	98	6.31 ± 1.57	3.37 ± 1.02	2.31 ± 0.63	0.30 ± 0.14	0.29 ± 0.23	116.1	59.6	226.3	1906
F	89	5.84 ± 1.57	3.12 ± 0.82	2.14 ± 0.45	0.28 ± 0.12	0.27 ± 0.22	88.3	43.1	158.2	1372
Both	187	6.09 ± 1.57	3.26 ± 0.93	2.23 ± 0.55	0.29 ± 0.13	0.28 ± 0.23				

Table 7

Coefficients of Correlations of Age and Packed cell volume (PCV) with total and differential leucocyte counts in 200 Indians

	*Multiple Regression *(P)*		Spearman's r *(P)*	
	AGE	PCV	AGE	PCV
Total Leucocytes	- .264 (.069)	.135 (0.056)	- .102 (.150)	.121 (.038)
Neutrophils	- .225 (.098)	.299 (.014)	.038 (.353)	.054 (.215)
Lymphocytes	.125 (.350)	.104 (.133)	- .069 (.245)	.107 (.058)
Monocytes	- .282 (.014)	.211 (.002)	- .299 (.001)	.173 (.006)
Eosinophils	- .153 (.026)	.194 (0.19)	- .181 (.004)	.112 (.050)

*Figures denote Multiple R. '-' sign denotes negative correlation, 'no sign' denotes positive correlation.

rence reached the level of significance only in the case of Indian females and European males ($p<0.01$).

Duration of stay of Europeans in India: (Table 5). The Europeans residing in India for 1 to 10 years were classified in two groups,

i) those who were living in India for less than five years and,

ii) those who were living in India for more than five years.

The average age of these two groups was 48.4 years in females. Males of the two groups had an average age of 43.5 and 45.6 years respectively. Subjects having a longer duration of stay showed a lower total leucocyte and neutrophil counts both in summer and winter. But the difference is significant only in the case of males in winter ($p<0.05$).

Diet: The total and differential leucocyte counts in 41 vegetarian and 187 non-vegetarian Indians are presented in Table 6. There was no significant difference of total and differential leucocytes counts in either sex in these two groups in spite of variable calorie and protein intake. The vegetarian males had a significantly higher count of eosinophils compared to that of non-vegetarians. However, there was no such difference in the case of females.

Physique: All the white cell counts were poorly correlated with four body-size variables namely, height, weight, surface area and ponderal index.

Blood groups: There was no significant association of total and differential leucocyte counts with ABO and Rhesus blood groups in either Europeans or Indians.

Correlation with other parameters: Multiple regression analyses showed no correlation of different leucocyte counts with haemoglobin concentration, total red cell count, red cell incices, erythrocyte sedimentation rate and different serum protein fractions (Table 7). However, a positive correlation was observed between packed cell volume and the number of monocytes ($p<0.01$) and eosinophils ($p<0.05$).

DISCUSSION

Ethnic variation: The findings show no significant difference in the total leucocyte count between the Europeans and Indians. South Indian male students had a higher total leucocyte count than the Europeans and North Indian students. Racial differences in total leucocyte counts have been reported among Negroes and Europeans in the United Kingdom and United States (1, 2, 5, 3, 4, 15). This difference was expressed as leucopenia due to neutropenia among the Negroes. However in studies in East and South Africa, no significant difference in leucocyte count was observed between Indians and Europeans living in Africa (6, 8). This is in agreement with the present observation. The number of the South Indians investigated was too small for any valid conclusion.

Age: The number of monocyte and eosinophil decreased with advancing age among the Indians in the present study. This observation was consistent in both the sexes separately and together, and in either seasons. This held true when the hypothesis of age dependence was tested both by multiple regression and Spearman's rank correlation tests (Table 7). There was an insignificant apparent decline of all the leucocytes with advancing age when the subjects of different age groups were compared (Table 2). This may be due to the confounding by other influencing factors which could not be ruled out by the standard tests available. In the literature there are conflicting reports of the age dependence of leucocyte counts. Zacharski et al (9) did not find any age dependence of the leucocyte counts in the American adults while MacKenney (12) reported a gradual decline of lymphocyte counts. They did not perform multiple regression tests on their results. Polednak (11) observed a significant positive correlation of age with the monocyte and eosinophil counts in both cross-sectional and longitudinal data. But his subjects were women of older age-groups working in Radium dial factory. They cannot be considered as a representative of the general healthy population. Munan and Kelly (13) reported a decline of the monocyte and eosinophil count in women. The present study was conducted under controlled condition among the healthy population of India and many variables were considered by stepwise multiple regression tests which might have some influence on the leucocyte counts. It will be interesting to carry out a similar study at a later date on the same population

to note the longitudinal changes in the leucocyte count and to verify this finding of the age-dependence of the monocyte and eosinophil counts.

Socio-economic status: Indian subjects of higher socio-economic status had a significantly higher count of monocyte than those of lower socio-economic status. Only female subjects of lower socio-economic status were available for comparison. These two groups were of comparable ethnic origin and age. This observation is in conformity with the observed positive correlation of monocyte count with the packed cell volume.

Seasonal variation: Seasonal variation of the total leucocyte count with an increase in the winter was seen in all the groups and both sexes. But the difference was significant only in case of Indian females and European males. Kennedy and MacKay (7) reported similar finding on the military personnel of the Royal Air Force of U.K. stationed in the middle-east and Africa. There was a fall in the total leucocyte count in July and August, with a reduction in the number of neutrophils. Sunlight, ultra-violet radiation and heat have been suggested as the causative agents for the increase in the lymphocyte count and a reduction in the neutrophil count (25, 7). The present finding of the seasonal variation of the leucocytes is in conformity with the above. In view of the insignificant increase of the number of leucocytes in the winter in European females and Indian males, a conclusion cannot be drawn from the present investigation. Probably a larger sample size will be necessary for confirmation of the seasonal influence on the leucocyte counts.

Duration of stay of Europeans in India: Europeans with longer stay in India showed a lower count of neutrophils than those with a shorter stay. There was no significant difference of age between these two groups. The difference was significant only in case of neutrophil counts among male subjects in winter. This may be due to the relatively small number of individuals studied. With the wide range of variation of the leucocyte counts, it is of course desirable to study large samples of individuals. However, our finding of lower leucocyte count in male Europeans with longer duration of stay in winter suggests an environmental influence on the leucocyte counts (4).

Diet: It was seen in the present study that the type of the diet did not have any significant influence on the leucocyte counts. It appears that the lack of animal protein intake is not an important determinant of the leucocyte counts because there is no significant difference between the vegetarian and non-vegetarian subjects. Trowell (26) also failed to find any evidence to support the view that malnutrition or undernutrition may cause neutropenia, which is in disagreement with the claim by Ezeilo (4) that dietary deficiency, particularly of animal protein, is the main cause of leucopenia among Africans.

Physique: In the present investigation four body-size parameters (height, weight, surface area and ponderal index) showed poor and inconsistent correlations with different leucocyte counts. In view of the conflicting reports of the relationship between physique and leucocyte counts in the literature it seems that there is little evidence for the existence of such a correlation (20, 11, 14).

Correlation between packed cell volume and leucocytes: The packed cell volume showed a consistent significant positive correlation with monocyte and eosinophil counts in this investigation. There was no significant correlation between these two leucocyte counts and the number of red cells, haemoglobin concentration or other red cell indices. The finding of a single positive correlation out of many tested relationships makes it necessary to draw conclusions with some caution. The packed cell volume is an indication of the general nutritional state of the body and the measurement is more reliable than other red cell indices. The observations of an age-dependent decline of monocyte and eosinophil counts and a higher monocyte count in subjects from higher socio-economic groups are compatible with the observed correlation between packed cell volume and the two leucocyte counts in question. It is also known that in older age groups and low socio-economic groups the packed cell volume is lowered. A lack of association between leucocyte counts and genetic markers is in agreement with the findings of Shaper and Lewis (2) in African subjects.

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