



## Comparative Study of Peripheral Smear with RBC Indices and RBC Histogram in Diagnosis of Anemia

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### ABSTRACT

**Title:** Comparative study of peripheral smear with RBC indices and RBC histogram in diagnosis of anemia.

**Aim:** To compare and correlate between diagnoses of anemia made by peripheral smear examination with RBC indices and RBC histograms.

**Methods:** 100 anemic patients having hemoglobin level of less than 11 gm/dl were taken into study. Complete blood count including hemoglobin, Total count, differential count, haematocrit value, RBC indices and histogram was obtained from Mindray BC 5300 automated analyser. Peripheral blood smear examination was done for every patient. Comparison between diagnosis by peripheral smear versus RBC histogram and indices was done.

**Results:** Out of 100 cases, 18% were normocytic normochromic anemia, 62% microcytic hypochromic anemia, 6% macrocytic anemia and 14% dimorphic anemia by peripheral smear examination. In normocytic normochromic anemia on peripheral smear, 89% showed normal curve in histogram. Left shift was seen in 73% of microcytic hypochromic anemia and all the curves were right shifted in macrocytic anemia. Dimorphic anemia showed variability where majority of the curve showed bimodal and left shift in histogram.

**Conclusion:** RBC histogram, indices and peripheral smear all are necessary for diagnosis of anemia and its types. Histograms along with RBC indices are supplementary to peripheral smear examination for correct diagnosis of RBC disorders.

**Keywords:** Histogram, RBC indices, peripheral smear, Anemia

### INTRODUCTION

Anemia is defined as a reduction in haemoglobin concentration below the level, which is expected for healthy persons of same age and sex and in the same environment. Adequate oxygen cannot be delivered to various organs and tissues due to low oxygen carrying capacity of the blood<sup>1</sup>. Manual blood smear examination along with RBC histogram and red cell indices is important to ensure the quality of blood count results and presumptive diagnosis of anemia. In

developing countries, anemia is a major global health problem. India is among the countries with high prevalence of anemia. As per new global nutrition report 2017- In India more than half (51%) of all women of reproductive age have anaemia<sup>2</sup>. Classification of anemia is based on parameters like haemoglobin, hematocrit, MCV(Mean cell volume), RDW(Red cell distribution width), MCH(Mean corpuscular volume) and MCHC(Mean corpuscular

haemoglobin concentration). These values are obtained by automated haematology analyzer and displayed as visual image in the form of RBC histogram. RBC histograms are graphical representation of cell frequencies versus cell size. RBC histogram show different pattern in various type of anemia which give clue in diagnosis of anemia. RBC histogram is an integral part of automated haematology analysis which has increased the accuracy and improved the precision.

Red cell indices and RBC histogram cannot identify condition such as red cell inclusions and membrane abnormalities<sup>3</sup>. Red cell indices like MCV which is a mean value does not reflect the heterogeneity of RBC population which can be identified by peripheral smear examination. So, for proper diagnosis of anemia RBC histogram, red cell indices along with peripheral smear examination are essential.

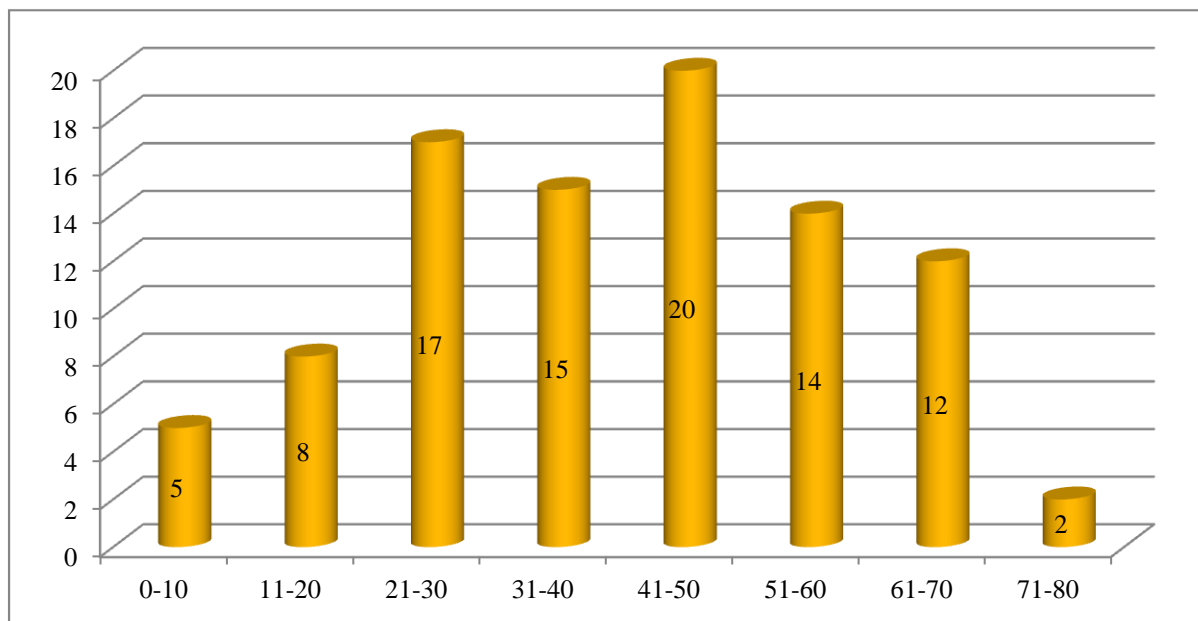
**MATERIAL AND METHODS:**

100 anemic patients having hemoglobin level of less than 11 gm/dl were taken into study. The present study was done in the central diagnostic laboratory of

Meenakshi Medical College and Research Institute over a period of 2017 to 2019. Patients with leukemia and pregnant women were excluded from the study. The present study is a diagnostic study. Complete blood count including hemoglobin, Total count, differential count, haematocrit value, RBC indices and histogram was obtained from Mindray BC 5300 automated analyser. Simultaneously, a Peripheral blood smear was prepared according to standard operating procedures and stained by Leishman stain, was done for every patient. Comparison between diagnosis by peripheral smear versus RBC histogram and indices was done by pearsons chi square test.

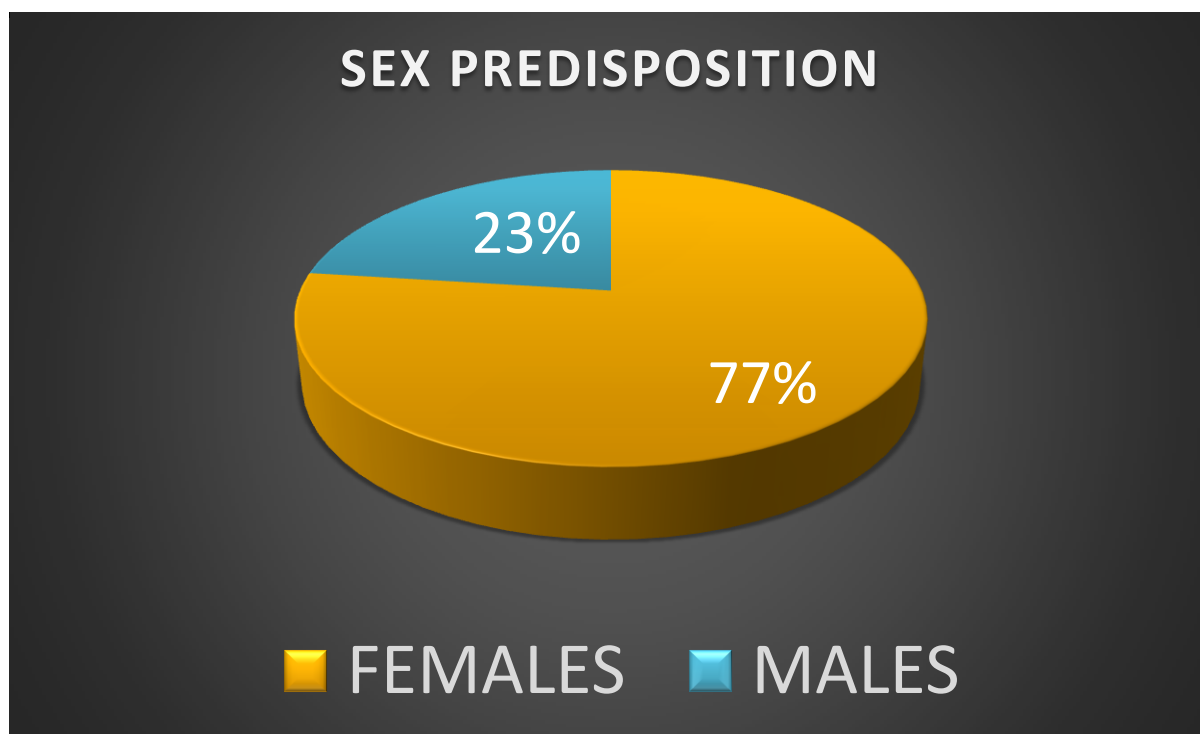
**RESULTS:**

The RBC histogram, red cell indices and peripheral smear of 100 patients having haemoglobin less than 11 gm/dl were analysed. The age group of the patients included in this study ranged from 2 to 80years. Majority of the cases were in the age group of 41-50 years (**Figure 1**).



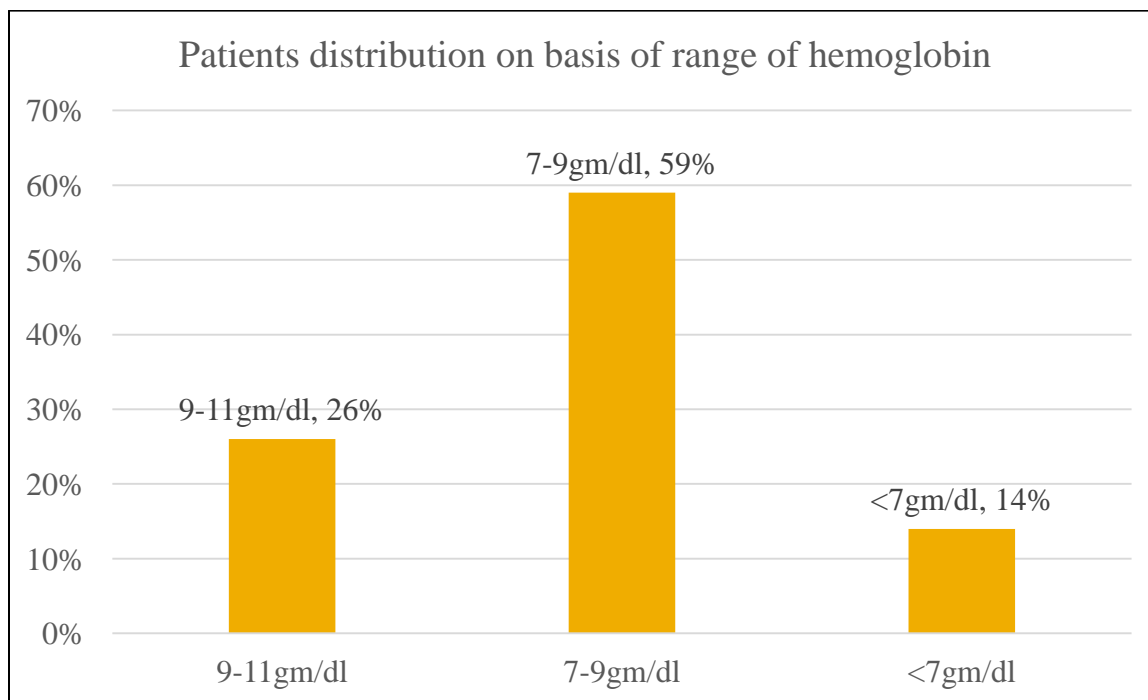
**Figure1: Age wise distribution of cases (X Axis – Age, Y Axis – Number of Patients)**

There was female preponderance. Out of 100 Patients, 77% were females and 23% were male (**Figure 2**).



**Figure2: Sex predisposition**

According to haemoglobin value, anemia was divided as per WHO criteria into mild (9-11gm/dl), moderate(7-9gm/dl) and severe(less than 7gm/dl). In our study 59% cases have haemoglobin in the range of 7-9gm/dl (**figure 3**). Mean haemoglobin was  $8.2\pm 2.3$ gm/dl. 53% cases were found to have microcytic hypochromic anemia on the basis of MCV value ( $MCV < 83$ fl), 37% cases showed normocytic normochromic anemia with MCV value between 83-101fl and 10% cases were diagnosed as macrocytic anemia with  $MCV > 101$ fl.



**Figure 3: Patients distribution on basis of range of haemoglobin**

Anemia when diagnosed on MCV values and manual examination of peripheral smear, the difference was statistically significant ( $p < 0.001$ ). This is due to dimorphic anemia where 64.3% of the cases were included in the normal range of MCV (**Table 1**).

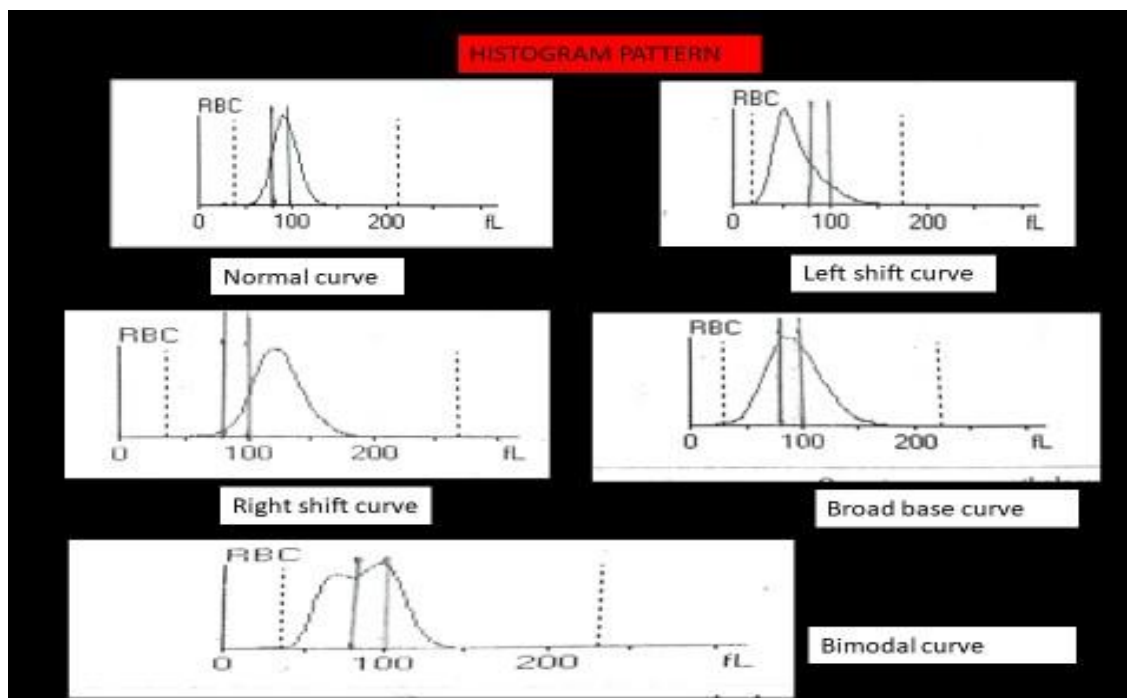
In the present study, 68% showed  $MCH < 27$  pg (considered as microcytic hypochromic anemia), 25% showed MCH value between MCH 27-31 pg (normocytic normochromic anemia) and 7% showed  $MCH > 32$  pg (macrocytic anemia).

Out of 62 cases of microcytic hypochromic anemia on peripheral smear, 56 cases (90%) of them had  $MCH < 27$  pg. Anemia when diagnosed on MCH values and manual examination of peripheral smear,

difference was statistically significant ( $p < 0.001$ ). (**Table 2**)

Majority of cases (81) showed  $MCHC < 315$  g/l. Anemia when diagnosed on MCHC values and manual examination of peripheral smear, difference was statistically significant ( $p < 0.001$ ). (**Table 3**)

Majority of microcytic hypochromic anemia cases showed left shift curve in histogram and all cases of macrocytic anemia showed right side curve while in dimorphic anemia, 35.71% showed bimodal curve and 21.43% showed broad base curve along with some cases showing normal curve and left shifting of curve (**Table 4, figure 4**).



**Figure 4: Histogram Pattern**

Out of 62 cases of microcytic hypochromic anemia diagnosed by peripheral smear, 85.4% showed  $RDW-CV > 14\%$ .

Anemia when diagnosed on histogram and manual examination of peripheral smear, difference was statistically significant ( $p < 0.001$ ).

All types of anemia correlated well with the histogram pattern except for dimorphic anemia, which showed different type histogram curve.

**DISCUSSION:**

In this study of anemic patients, peripheral blood smear examination were evaluated and compared with histogram and red blood cell indices of 100 patients.

It was seen that out of 100 cases, majority of case fall in the adult age 41-50 years. This can be explained as a period of adolescent and adult group is period of intense growth and development. Iron is in high demand because it is involved in fundamental basic physiological process such as haemoglobin formation.

The results were in concordance with the study conducted by Japheth et al<sup>4</sup>, Kumar et al<sup>5</sup>, cook et al<sup>6</sup>. Regarding distribution of sex in this study, 77 were female and 23 were male which shows there was female preponderance. Out of 77 females majority were in the reproductive age group. Women in reproductive age group are at high risk of developing anemia because of rapid growth and frequent loss of blood during menstruation.

Based on WHO criteria, we considered all the cases with haemoglobin < 11 gm/dl. The mean haemoglobin was 8.2±2.3 gm/dl. Majority (59%) have haemoglobin in the range of 7-9 gm/dl. Patel et al<sup>7</sup> and Kumar et al<sup>5</sup> studies also showed mean haemoglobin as 7.2 gm% and 5.85 gm% respectively.

Most common morphological type of anemia was microcytic hypochromic anemia (62%) followed by normocytic normochromic anemia (18%). In the world, India is one of the countries with very high prevalence of anemia. Most common cause of anemia in developing countries among vulnerable groups like pregnant women are nutritional disorders (iron deficiency anemia) and infections. About 58% of pregnant women in India are anaemic and anemia is the underlying cause for 20-40% of maternal deaths in India. India contributes to about 80% of the maternal deaths due to anaemia in south Asia<sup>8</sup>.

Out of 100 cases, 53% cases shows MCV < 83 fl, 37% cases shows normal MCV and 10% cases shows MCV > 101. This is in concordance with the study conducted by Patel et al.<sup>7</sup> In Present study majority (68%) of patients show MCH < 27 pg which is similar to study conducted by Patel et al<sup>7</sup>. Majority of cases with microcytic hypochromic anemia shows high RDW<sup>9</sup>.

Histogram is generated by cell counters, which are graphical representation of different population cell types and are neglected piece of information by hematopathologist.<sup>10</sup> Histogram in association with RBC indices and peripheral blood smear is found abnormal in various haematological condition<sup>11,12,13</sup>. In present study 100 patient of anemia were analyzed and comparison between peripheral smear report and RBC indices with RBC histogram obtained from Mindray BC5300 automated analyzer was done. For better understanding of histogram, a dotted line depicting a reference normal curve was drawn against every red cell histogram to show variations of

histogram in various disease condition. In present study, various type of anemia that are microcytic hypochromic anemia (62%), normocytic normochromic anemia (18%), macrocytic anemia (6%) and dimorphic anemia (14%) were diagnosed on peripheral smear. We found that in smear reported as microcytic hypochromic anemia, 73% histogram showed left shift because of small size of RBCs. Peripheral smear having normocytic normochromic anemia showed 89% normal curve and all the smear with macrocytic blood picture showed right shift of histogram pattern. Our study was in concordance with the study conducted by Constantino et al<sup>14</sup>. Seeing this comparison histogram are useful diagnostic aid in case of normocytic normochromic anemia, microcytic hypochromic anemia and macrocytic anemia. In dimorphic anemia, histogram showed different pattern from simple to complex curve. Majority 35.71% showed bimodal curve, 21.43% showed broad base curve and only 14.28% showed left shift curve and 28.57% showed normal curve. Different histogram patterns have been described to categorize dimorphic anemia<sup>15</sup>. The significant difference was largely due to dimorphic anemia cases. Bimodal curve can be explained by presence of multiple population of cells of varying size that is normocytic, microcytic and macrocytic. Bimodal histogram not only is associated with therapeutic transfusion and hematinic agent response to microcytic and macrocytic anemia but it also indicates further hematological disorder such as early iron developing microcytic population, folate vitamin B12 developing macrocytic population, post iron treatment of iron deficiency anemia, post iron treatment of iron deficiency with megaloblastic anemia.<sup>16</sup> The centre and the width in the histogram of dimorphic anemia shows the variation in the RBC. Dimorphic blood picture is dual population of microcytic and normocytic or normocytic and macrocytic red cell or mixture of small, normal or large cell of different size and forms with or without normal red blood cell indices which can mislead the diagnosis if we rely on automated values alone. So, it is very important to examine peripheral blood smear, red blood cell indices and histogram in all patients of anemia.

In the present study we also classified 100 cases of anemia based on red blood cell indices (MCV, MCH, MCHC). These findings were compared with



peripheral smear examination as the gold standard. MCV<83fl were considered microcytic, 83-101fl were considered normocytic and dimorphic and more than 101fl were considered macrocytic. MCH <27pg were consider microcytic, 27-32pg were consider normocytic or dimorphic and >32pg were consider as macrocytic. MCV represent only mean of distribution curve and insensitive to small number of microcytes and macrocytes. Probable reason may be MCV is an average value and doesnot reflect the presence of different red cell population<sup>17,18</sup>. Therefore in all the patients of anemia, peripheral smear examination is must in identifying early red blood cell changes. In our study, majority of cases (81%) showed MCHC <315 g/l because in anemic patients red blood cells are deficient in haemoglobin suggested by low MCHC value. MCH, MCHC give little information independent of MCV. In present study we noticed that majority of microcytic hypochromic anemia (85.4%) shows high RDW, thus confirming that RDW is a sensitive indicator of anisocytosis.<sup>19</sup> RDW also show better correlation as an indicator of anisocytosis when MCV is in low normal range, like in case ofdeveloping iron deficiency. Red cell histogram in patient of iron deficiency anemia shows left shift but on treatment histogram widens consequently due to increased in RDW. There is increase in red cell heterogeneity producing more abnormal cell in peripheral blood in nutritional deficiency(iron, folate, vitamin B12).

Very few study have focused on RBC histogram while giving more significance to WBC histogram. Our study revealed an important correlation between RBC histogram and peripheral smear examination for diagnosis in microcytic hypochromic anemia, normocytic normochromic anemia and macrocytic anemia. However, the relationship between histogram pattern, red cell indices and peripheral smear examination in a diagnosis of dimorphic anemia posed some query regarding validity of RBC histogram.

It can be concluded from our study that in the age of molecular analysis and automation, peripheral smear examination along with clinical history is an important diagnostic tool while dealing the patient of anemia. Only RBC histogram or red cell indices are not sufficient to diagnose the case of different types of anemia. They can only aide to visual examination of peripheral smear.

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**TABLES:**

**Table 1: Correlation of MCV and peripheral smear**

		Peripheral Smear				Total
		Normocytic Normochromic	Microcytic Hypochromic	Macrocytic Anemia	Dimorphic Anemia	
MCV	Normocytic Normochromic	14	14	0	9	37
	Microcytic Hypochromic	0	48	0	5	53
	Macrocytic	4	0	6	0	10
Total		18	62	6	14	100

**Table 2: Correlation of MCH and peripheral smear finding**

		Peripheral Smear				Total
		normocytic normochromic	microcytic hypochromic	macrocytic anaemia	dimorphic anemia	
MCH	normocytic normochromic	13	6	1	5	25
	microcytic hypochromic	3	56	0	9	68
	macrocytic	2	0	5	0	7

Total	18	62	6	14	100
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**Table 3: Correlation of MCHC and peripheral smear finding**

		Peripheral Smear				Total
		normocytic normochromic	microcytic hypochromic	macrocytic anaemia	dimorphic anemia	
MCHC	normocytic normochromic	6	7	2	3	18
	microcytic hypochromic	12	55	3	11	81
	macrocytic	0	0	1	0	1
Total		18	62	6	14	100

**Table 4: Correlation of peripheral smear and histogram**

		Histogram					Total
		normal curve	right shift curve	left shift curve	broad base curve	bimodal curve	
Peripheral Smear	normocytic normochromic	15	2	0	1	0	18
	microcytic hypochromic	10	0	45	6	1	62
	macrocytic anaemia	0	6	0	0	0	6
	dimorphic anemia	4	0	2	3	5	14
Total		29	8	47	10	6	100