

# Comparative Study of Mean Corpuscular Volume Between Lacto-vegetarian and Non-vegetarian Populations of Tharparkar Village

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## ABSTRACT

**Objective:** Anaemia caused due to micronutrient deficiencies is commonly found in our country. Mean Corpuscular Volume (MCV) is the average volume of red cells and largely depends upon micronutrients like B12, folic acid, and iron present in diet. Deficiency of these micronutrient, affects MCV and can cause anaemia. This study aimed to compare the MCV of lacto-vegetarian and non-vegetarian adults living in Tharparkar-Sindh village.

**Methods:** Case control analytical descriptive study. One hundred apparently healthy strict lacto-vegetarian and non-vegetarian subjects were selected from the same village of Tharparkar. After written consent and preliminary physical examination, blood samples were collected under sterilized condition in two tubes—one containing EDTA for CBC and the second for serum for B12 and folate. Peripheral smear was made at the research field site and fixed with 70% methanol to maintain cellular morphology. Samples were analyzed for complete blood count, serum B12, and folate.

**Results:** The mean age in vegetarian group was 30.5 years ( $\pm 8.36$ ) and 30.13 ( $\pm 9.22$ ) in non-vegetarian group. Male to female ratio was 3.4:1. On direct questioning, 54 vegetarians and 24 non-vegetarians agreed to have felt fatigue and lethargy. Most of the subjects in both groups relied on wheat, pulses, vegetables, and milk products as staple food whereas non-vegetarians often consumed eggs and meat as well. In vegetarian group, 83% and in non-vegetarian group, 66% subjects were found to be B12 deficient, while 7% vegetarians and 23% non-vegetarians had low folate level. Nine vegetarians and 22 non-vegetarians were found to be anaemic. A total of 20 vegetarian subjects and 4 non-vegetarian subjects were found with definite high MCV i.e. more than 100.

**Conclusion:** Majority of the vegetarians and more than half of the non-vegetarians had vitamin B12 deficiency while folate levels were normal in most of the subjects of both groups. MCV is a poor indicator of the severity of B12 and folate deficiency anaemia and normal MCV does not exclude B12 or folate deficiency. Clinicians need to be aware of the low sensitivity of the MCV while screening.

**Key words:** Anaemia, MCV, Lactovegetarian, Macrocytic

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عنوان: تھر پارکر کے گاؤں کی آبادی کے گوشت خور اور مکمل سبزی خور افراد میں Mean Corpuscular Volume کا جائزہ۔

تعارف: خون کی کمی ہمارے معاشرے میں ایک عام مرض ہے۔ Mean Corpuscular Volume (MCV) ہماری خوراک میں شامل اجزاء جیسے وٹامن B12 آئرن اور فولک ایسڈ پر منحصر ہے۔ ان اجزاء کی کمی خون کی کمی کا باعث ہوتی ہے۔ اس تحقیق کا مقصد تھر پارکر کے گاؤں میں سبزی خور اور گوشت خور افراد کا MCV کا موازنہ کرنا ہے۔

طریقہ کار: تھر پارکر کے ایک ہی گاؤں سے بظاہر صحت مند نظر آنے والے 100 مکمل سبزی خور اور گوشت خور افراد منتخب کیے گئے۔ اور ان کی رضامندی سے حفظان صحت کے اصولوں کو مدنظر رکھ کر 2 شیفٹس کی ٹالیوں میں خون کے نمونے لیے گئے۔ ایک ٹیوب کی مدد سے CBC اور دوسری کی مدد سے وٹامن B12 اور folate کی مقدار کا جائزہ لیا گیا۔ نمونوں کی خلیاتی صورت محفوظ رکھنے کے لیے فیلڈ پر ہی 70 فیصد میتھانول استعمال کی گئی۔ اور ان کی CBC، وٹامن B12 اور folate کی جانچ کی گئی۔

نتیجہ: سبزی خور گروہ کی اوسط عمر 30.5 سال ( $\pm 8.36$ ) اور گوشت خور گروہ کی اوسط عمر 30.13 سال ( $\pm 9.22$ ) تھی۔ جبکہ مرد اور عورت کا تناسب 3.4:1 تھا۔ سوال کرنے پر 54 سبزی خور اور 24 گوشت خور افراد نے بتایا کہ انھیں تھکن اور سستی محسوس ہوتی ہے۔ دونوں گروہوں میں زیادہ تر افراد کی خوراک میں گندم، چاول، دالیں، سبزیوں اور دودھ شامل تھا جبکہ گوشت خور افراد انڈے اور گوشت کا بھی استعمال کرتے تھے۔ سبزی خور گروہ 83% اور گوشت خور گروہ میں 66% افراد وٹامن B12 کی کمی کا شکار پائے گئے۔ جبکہ 7% سبزی خوروں اور 23% گوشت خوروں میں Folate level بھی کم پایا گیا۔ 9 سبزی خور افراد اور 22 گوشت خور افراد خون کی کمی کا شکار تھے۔ اسی طرح 20 سبزی خور افراد اور 04 گوشت خور افراد شدید MCV (100 سے زیادہ) کا شکار تھے۔

حاصل مطالعہ: سبزی خوروں میں اکثریت اور گوشت خوروں میں آدھے سے زیادہ افراد وٹامن B12 کی کمی کا شکار تھے جبکہ دونوں گروہوں میں folate level نارمل تھا۔ MCV وٹامن B12 کی کمی اور خون کی کمی کے لیے بری علامت ہے۔ اسکریپنگ کے وقت ڈاکٹروں کو MCV کی حساسیت کا خیال رکھنا چاہیے۔

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## INTRODUCTION

Tharparkar is a neglected district of the province of Sindh, inhabited by two communities living together for centuries. One of these communities is lacto-vegetarian and the other is non-vegetarian. The lack of basic facilities, education and some religious customs, have placed the population of this area at a high risk of dietary deficiency. Anaemia is defined as a clinical condition characterized by haemoglobin concentration below normal for age, sex, physiological condition, and altitude above the sea level<sup>1</sup>. It is a global concern affecting poor populations in the developing countries mainly due to micronutrient deficiency in their diet. Lack of knowledge of the dietary sources of B-complex vitamin, ignorance of the importance of these vitamins' daily consumption and poor socioeconomic conditions are major contributors of low biochemical levels of these essential nutrients in Pakistani population.

Among other micronutrients, vitamin B12 and folate play the most important role in the maturation of blood cells and stability of neuron cells<sup>2</sup>. Severe and irreversible damage of brain and neuron system are reported to be due to vitamin B12 and folate deficiency<sup>3</sup>.

Vegetarians diet is vitamin B12 deficient because the major source of this essential vitamin is animal products while it is found in a negligible amount in dairy product. Hence, vegetarians are found to be more deficient in vitamin B12<sup>4</sup>. On the other hand, folate can be found in both animal and plant sources and its deficiency has been rarely reported<sup>5</sup>. Deficiency of vitamin B12 and folate leads to macrocytic anaemia<sup>6</sup>.

Vitamin B12 and folic acid are essential dietary components for humans, because they are required for DNA synthesis. Hematopoietic cells are especially sensitive to deficiencies of folate and vitamin B12. With derangement of DNA synthesis due to deficiencies of these vitamins, megaloblastic anaemia ensues<sup>7</sup>. Vitamin B12 and folate deficiencies are quite common among Pakistani individuals and may be a leading cause of megaloblastic anaemia in our population<sup>8</sup>.

Mean Corpuscular Volume (MCV) is the average volume of red cells. In specimen, MCV is elevated or decreased in accordance with average red cell size i.e. normal MCV indicates normocytic, low MCV indicates microcytic, and high MCV indicates macrocytic anaemia. This is used as a morphological basis of anaemia<sup>9</sup>. The reference range for MCV is 80-95 fl in adults<sup>10</sup>. Macrocytosis is generally defined as an MCV greater than 100fl.

MCV largely depends upon micronutrients like B12, folic acid, and iron present in diet. Deficiency of these

micronutrients affects MCV and can cause anaemia<sup>11</sup>. When the peripheral smear indicates megaloblastic anaemia, the most likely cause is vitamin B12 or folate deficiency<sup>12</sup>. Macrocytosis occurs in approximately 3% of general population worldwide<sup>13</sup>. Megaloblastic anaemia is frequently observed in clinical practice in Pakistan. However, this paper is based on a unique comparative study of MCV in two communities only differing in their dietary pattern.

## METHODOLOGY

This descriptive cross-sectional study was conducted on n=100 lacto-vegetarian and n=100 non-vegetarian apparently healthy subjects of both genders, in the rural area of Tharparkar in 2012. The study was conducted after receiving ethical approval from the ethical committee of the Dow University of Health Sciences. Subjects were introduced to this project at a local assembly place. Informed consent was taken before enrolling the participants in the study. The literacy rate in this population was 35 % so the project was explained in detail to the uneducated subjects and their thumb impressions or signatures were obtained on the consent form. The subjects aged between 14 and 55 year. Those with strict lacto-vegetarian diet, were grouped as group 1 and non-vegetarians of the same age were included in group 2. Apparently healthy subjects were included and those who were taking multivitamin preparations orally or parenterally, subjects with history of blood transfusion, history of diarrhoea, worm infestation and those aged less than 14 years were excluded from study. After taking aseptic measures, anti-coagulated whole blood samples were collected for assessing multiple parameters of blood i.e. Complete Blood Count (CBC) for Mean Corpuscular Volume (MCV), vitamin B12 and folate level.

Peripheral blood smear was made at the research field site and fixed with 70% methanol, so that cellular morphology could be maintained. The sample slides and tubes were coded with the subject's serial numbers and full names. After collection of required quantity, the samples were stored in two iceboxes having 4°C and 0°C temperatures. Samples were safely transported within 8–10 hours to Dow Diagnostic Research Laboratory, Karachi for analysis using an automated cell counter TAC-alpha (5 parts) for CBC and serum B12 and folate levels. The peripheral blood smear was stained with Leishman stain. Peripheral smear morphology was observed by using conventional microscopy.

MCV between lacto-vegetarian and non-vegetarian populations

Table 1. Comparison of the Mean Value of Serum B12 and Folate Level in Both Groups Using Independent Sample T-test

Group		Deficiency	Mean	SD	P-Value
Vitamin B12 Deficiency<205	Vegetarian n-100	82	147.29	31.78	<0.01*
	Non-Vegetarian n-100	66	127.7	42.71	
Folic Acid Deficiency<2.6	Vegetarian n-100	7	6.72	2.70	<0.01*
	Non-Vegetarian n-100	23	4.41	2.79	

\*p<0.05 was considered significant using independent sample t-test

Table 2. Peripheral Smear Morphology Patterns in Research Population

Gender	Morphology	Vegetarian		Non-Vegetarian	
		n	%	n	%
Male	Normocytic/Normochromic	52	76.5	75	94.9
	Hypochromic/Microcytic	1	1.5	1	1.3
	Macrocytic	15	22.1	3	3.8
Female	Normocytic/Normochromic	27	84.4	16	76.2
	Hypochromic/Microcytic	3	9.4	5	23.8
	Macrocytic	2	6.2	0	.0

Table 3. Comparative Analysis of MCV in Both Groups

MCV	Groups			
	Vegetarian		Non-vegetarian	
	n	%	n	%
55 -85	9	9.0	39	39.0
85 - 100	71	71.0	57	57.0
>100	20	20.0	4	4.0

\*p<0.05 was considered significant using Pearson Chi Square test

The reference ranges for both the genders were used in accordance to the standard guidelines<sup>14</sup>. For morphology, normochromic normocytic picture was considered as non-anaemic. Hypochromia with poikilocytosis and anisocytosis as either iron deficiency or haemoglobinopathy or both; while macrocytosis was taken as vitamin B12 or folic acid deficiency anaemia. Out of two approaches, i.e. kinetic (focusing on production, destruction, and loss) and morphological (based on RBC size), the latter was used as conventionally done by routine haematology analyzer.

## RESULTS

The mean age in vegetarian group was 30.5 years ( $\pm 8.3$ , n=100) with male to female ratio 2.1:1. In non-vegetarians, the mean age was 30.13 years ( $\pm 9.3$ , n=100) and male to female ratio was 3.4 :1. The literacy rate

was 35% in vegetarian group and while it was 22% in non-vegetarians. Common symptoms reported by subjects were weakness and fatigue at 54% in vegetarians and 24% in non-vegetarians. Mean haemoglobin found in vegetarians was 13.5 ( $\pm 3.2$ ) and 13.3 ( $\pm 2.4$ ) in non-vegetarians. A total of 9 subjects were found to be anaemic in the vegetarian group and 22 were found to be anaemic in the non-vegetarian group. MCV was measured and data showed that 9 vegetarian subjects and 39 non-vegetarian subjects had MCV < than 85 fl; 20 vegetarian subjects and 4 non-vegetarian subjects showed MCV values > 100 fl. A total of 83% of the vegetarians and 66% non-vegetarians were found to be vitamin B12 deficient i.e. > 205 ng/ml. However, only 7% of vegetarians were found to have folate deficiency compared to non-vegetarians who showed 23% folate deficiency i.e. < 2.6 ng/ml.

The mean B12 and folate level in vegetarians was 190.24 and 6.73, and 226.71 and 13.9, respectively in non-vegetarians (Table 1).

## DISCUSSION

The present study is unique in the sense that (a) to my knowledge, it was conducted for the first time in a rural area of Pakistan which is inhabited equally by the Muslims and the Hindus; (b) It focused on the principle of strict vegetarianism which is the chief feature of the studied Hindu population and non-vegetarians which are the Muslim population; (c) It explored the consequences of vitamin B12 and folate deficiencies on MCV of both groups.

In our study, the mean age of the vegetarians who were found to be vitamin B12 deficient was 30 years, this is different from the study done by Hashim and Tahir in Pakistan in 2006 who found the mean age to be 55 years<sup>14</sup>. In this study, male to female ratio was 2.1:1 in vegetarians and 3.7:1 in non-vegetarians. This difference in genders is due to the practice of veiling of the female population in Tharparkar village. Although all subjects (vegetarian and non-vegetarian) recruited in the study were apparently healthy and did not present any signs and symptoms, but on direct questioning, 54% vegetarians reported history of fatigue and weakness, while 24 % of non-vegetarians reported having unexplained lethargy. These findings are similar to the study done by Stable and colleagues in 1990<sup>15</sup>. These workers suggested that fatigue and lethargy are the most initial symptoms experienced by those who developed macrocytic anaemia. The mean B12 levels were found to be 190.2 (normal 205 ng/ml and above) in vegetarians and 226.7 in non-vegetarians which also showed a preponderance of B12 deficiency among the vegetarian group. The correlation coefficient between B12 and age was positive i.e. 0.173 with significant p value 0.014.

In case of folate level analysis, we also found the most expected results, like only 7% subjects in the vegetarian group and 23% subjects in non-vegetarian group were deficient in folate, with a highly significant p-value less than 0.0001. The mean folate levels were 6.7 ng/ml (normal above 2.6 ng/ml) and 13.9 ng/ml respectively. The correlation co-efficient between folic acid and age was negative i.e. 0.027 with an insignificant p-value 0.70. The prevalence of vitamin B12 deficiency in the present study is much higher than other studies while folate deficiency is also significantly on the higher side.

MCV is the most sensitive index in diagnosis of vitamin B12 /folate deficiency<sup>16</sup>. The degree of anaemia varies but macrocytic and hyper-segmented neutrophils are

considered important laboratory findings in many studies<sup>17</sup>. In our studies, we also observed early changes in RBC morphology. The blood films of most of the studied population were found to be normocytic and normochromic (Table 3). These findings matched with the study done by Khanduri and Sharma in 2007<sup>18</sup>. We found that MCV in vegetarian population was 93.6 ( $\pm 9.9$ ) and 87.6 ( $\pm 8.2$ ) in non-vegetarians. Maximum range was 118 fl. Seventy-one vegetarians and fifty-seven non-vegetarians had MCV between 85–100 fl in vegetarians with p-value less than 0.001. The most probable cause of borderline increase or definite increase in MCV in vegetarians is vitamin B12 or folate deficiency. This ecological influence on MCV was discussed in the study done by Graves and Lee in 2012<sup>19</sup>.

Another intricacy regarding the importance of MCV in vitamin B12/folate deficiency states is addressed in the study done by Spirak in 1982<sup>20</sup>. It says that if iron deficiency and thalassemia coexist with vitamin B12 and folate deficiency, than MCV will not be increased and may decrease in spite of vitamin B12 /and folate deficiency<sup>21</sup>. MCV can increase in many conditions such as in alcoholism, hypothyroidism, liver disease, pregnancy, and reticulocytosis so, we suggest excluding all these condition before labeling and deciding on vitamin B12/folate deficiency<sup>21</sup>. In our study, we had excluded notable conditions just indicated and we found increased MCV and serum vitamin B12/folate deficiency in 20% subjects in vegetarian group and only 4% in non-vegetarian group. Such poor correlation has been reported in the study done by Ward in 2002<sup>23</sup>.

## CONCLUSION

The vegetarian and non-vegetarian populations of Tharparkar district are sailing in the same boat because of poor economy, harsh environmental and sociocultural conditions. In this study, both groups exhibited severe vitamin B12 deficiency predominately in the vegetarian group, but that was not truly reflected from MCV. It seems to be a poor indicator of the severity of B12 and folate deficiency anaemia, hence, normal MCV does not exclude B12 or folate deficiencies. Clinicians need to be aware of the low sensitivity of the MCV for screening and the upper limit of MCV should not be more than 95 fl.

**Authors' contributions:** Dr Suresh Kumar conceived the idea, worked on literature search, data collection, data analysis and review, introduction and discussion. Dr Asma Sheikh and Dr Zareen Irshad worked on literature search, results and discussion. Dr Vinita kumari and Dr Salma Parween reviewed the literature,

worked on discussion and edited the manuscript. All authors discussed the results and contributed to the final manuscript.

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