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Red Cell Mass Measurement Using Technetium ^{99m}Tc to Differentiate Absolute Erythrocytosis (Polycythemia) from Relative Erythrocytosis

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Objective: The aim of this study is to show that measurement of red cell mass is essential to differentiate absolute erythrocytosis from relative (spurious) erythrocytosis.

Setting: Nuclear Center, Baghdad Teaching Hospital, Iraq.

Design: **Prospective study.**

Method: This study was performed in September 2002. Seven patients with packed cell volume over 51 were included in the study. Amerscan Stannous agent was injected intravenously followed by the collection of 10ml of blood at 15 minutes. 2 MBq (50uCi) of freshly generated ^{99m}Tc in 0.2ml of saline was added to the collected blood. A standard sample was retained for control. The suspension of ^{99m}TC was injected intravenously and blood sample was collected at 10, 20 and 30 minutes.

Radioactivity was measured using a scintillation counter. Red cell mass was calculated and then factored into an equation which included body weight, height and surface area.

Result: Three out of seven (42.85%) had increased red cell mass which indicates that PCV alone is not an accurate indication of true erythrocytosis. Patient's weight ranged from 52-90kg, the mean is 77.47kg and the surface area ranged 1.52-2.10 the mean is 1.90. Therefore, considering the surface area in the measurement of red cell mass is recommended.

Conclusion: **PCV alone is not accurate indication of true erythrocytosis. Surface area should be considered with the measurement.**

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Measurement of red cell mass (RCM) and its relationship to that expected for an individual's height and weight permits initial subdivision of erythrocytosis into absolute increased RCM or apparent normal RCM[1,2].

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A study conducted in Sweden showed a wide variation in the measurement of (RCM) performed on 61 of 62 (98%) patients in University Hospitals compared with 24 of 67 (36%) patients at country hospitals[1].

Plasma volume is influenced by bed rest, exercise, and change in posture, food and ambient temperature. Fluctuation in plasma volume may result in hemodilution or conversely in hemoconcentration, giving rise to pseudoanemia or pseudopolycythemia respectively[2]. A raised PCV (>0.51 in males, >0.48 in females) needs to be confirmed on a specimen taken without venous occlusion. Patients with persistently raised PCV should be referred to a hematologist for measurement of red cell mass by radionuclide labeling of the red cells[3]. However, when the Hematocrit (PCV) was ≥60 an absolute erythrocytosis was always present, and this was true for both male and female subjects[4].

According to the Polycythemia Vera study group (PVSG) criteria and revised criteria for the diagnosis of PV as proposed by Pearson and Messinezy in 1996 (PM criteria) an increment of RCM above 35 ml/kg in male and 30 ml/kg in females is an absolute criterion[3]. It was shown clearly that predictions based solely on body weight were inappropriate, particularly because approximately half of the male and female populations could be regarded as over-weight or obese[5]. Red cell mass is best expressed as the percentage difference between the measured value and that predicted from the patient's height and weight (derived from tables)[3]. Red cell mass more than 25% above the predicted value constitutes real or absolute Polycythaemia[3].

There are many methods for measurement of blood volume but the most practical method now available is to use a small volume of the patient's red cells labeled with radioactive chromium (^{51}Cr), technetium (^{99m}Tc) or indium (^{113m}In or ^{111}In) which is injected intravenously and it binds to the red cells and its dilution is measured after time has been allowed for the injected material to become thoroughly mixed in the circulation, but before significant quantities have left the circulation[2].

Absolute erythrocytosis may be primary (intrinsically abnormal marrow erythropoiesis) or secondary (increased erythropoietin drive in response to pathological events outside the bone marrow)[6]. The other criteria for diagnosis of Polycythemia Vera (PV) such as serum erythropoietin, endogenous erythroid colony formation (EEC), and JAK2 V617F gene have high specificity, but the sensitivities are not high enough to detect the early stages of PV and other myeloproliferative disorders MPDs[7].

The aim of this study is to demonstrate that measurement of RCM is essential to differentiate absolute erythrocytosis from relative erythrocytosis.

METHOD

The study was performed in September, 2002 at the Nuclear Medicine Centre in Baghdad. Informed consent, full medical history and clinical examination were obtained. Full precautions for sterilization and radiation exposure were employed. The surface area was calculated as a composite score of height and weight for all patients enrolled in the

study.

We dissolved a vial of Amerscan Stannous agent in 8ml of sterile saline, 4 ml of which was then injected intravenously. After 15 minutes, we collected 10ml of blood into a sterile container to which 200 IU of liquid heparin was added. The sample was centrifuged and washed twice with sterile saline to remove the extra cellular tin. Then we added 2 MBq (50uCi) of freshly generated ^{99m}Tc in approximately 0.2ml of saline which was then allowed to stand at room temperature for 5 minutes. The sample was centrifuged and washed twice in cold sterile saline and re-suspended in a sufficient volume of cold sterile saline up to 10ml[2].

The suspension was injected intravenously and immediately followed by flushing through with 10ml of sterile saline. At 10, 20 and 30 minutes intervals we collected 10ml of the patient's blood from a vein other than that used for the injection. The blood is kept in the appropriate EDTA tube.

We measured the PCV of each sample and then delivered 1ml volumes into counting tubes which were lysed with saponin by adding 2 drops of Saponin (Coulter); finally, we measured their radioactivity in a scintillation counter. Then we diluted the residue of the original suspension and injected in 500ml of water (to be used as a standard).

We determined the radioactivity of one 1ml volume using the equation, see Figure 1.

$$RCV = \frac{I}{C} = \frac{S \cdot D \cdot V_i}{C} = \frac{S \cdot D \cdot V_i \cdot H_v}{B}$$

where I = total amount of injected radioactivity (c/min)
C = concentration of radioactivity in red cells of blood sample drawn after mixing is completed (c/min per ml red cells)
S = concentration of radioactivity in diluted standard (c/min per ml)
D = dilution of diluted standard solution, i.e., final volume divided by volume of red cell suspension put into it
V_i = volume of labeled red cell suspension injected (ml)
H_v = PCV of whole blood sample corrected for trapped plasma
B = concentration of radioactivity in blood sample drawn after mixing is completed (c/min per ml blood)

Figure 1: Equation Used for Measurement Red Cell Mass

In two cases; A and C, collection was done at 60 minutes because the participants had splenomegaly.

RESULT

Red cell mass measurement using ^{99m}Tc was done for seven patients; one female and six males with PCV (hematocrit) of above 51, a range of PCV 52-56 and the mean of PCV was 53.42, weight ranged 52-90kg, a mean 77.47 kg and the surface area ranged 1.52-2.10 M^2/kg, a mean of (1.90) M^2/kg.

After measuring red cell mass and dividing the value by weight it was shown that there was a true increase in red cell mass in three patients (A, B and C) out of seven; two males and one female, patient A 43 ml/kg, patient B 36 ml/kg and lastly patient C 33.4 ml/kg.

The results for the remaining four patients (D, E, F and G) showed no increment in red cell mass as follows:

Patient D 30 ml/kg, patient E 27 ml /kg, patient F 23 ml /kg and patient G 26 ml /kg, see Table 1.

Table 1: Red Cell Mass Measurement in Seven Patients Using RCM, Ml/Kilograms Unit

Patients	Sex	PCV	RCM, mL	WT, kg	RCM,ml/kg
A	male	56	2230	52	43
B	male	54	3098	84	36
C	female	54	2417	72.3	33.4
D	male	53	2569	84	30
E	male	52	2049	75	27
F	male	53	2079	90	23
G	male	52	2234	85	26

After considering height and weight and measuring surface area for patients and comparing it to the means of the measurements done by Hurley, an increased red cell mass percentage in comparison with average similar surface area was noted (See Table 2)[2]. Patient A 131%, patient B 143% and patient C 153%, whilst no increase was noted in the other four patients. (Patient D 119%, E 100%, F 85% and G 92%). Although all patients included in the study had a hematocrit (PCV) above 51, only three of them had true erythrocytosis.

Table 2: Red Cell Mass Measurement in Seven Patients, Using Percentages of RCM to Average Mean of the Correlated Surface Area Meter/Square Weight

Patients	PCV	Wt, kg	Ht , cm	SA m^2/kg	RCM, ml	Average*, ml	Percentage**
A	56	52	161	1.52	2230	1699	131%
B	54	84	167	1.95	3098	2156	143%
C	54	72.3	165	1.76	2417	2417	153%
D	53	84	165	1.95	2569	2156	119%
E	52	75	173	1.97	2049	2029	100%
F	53	90	180	2.10	2079	2463	85%
G	52	85	178	2.08	2234	2419	92%

* Red cell volume in normal adults according to surface area by HURLEY, P.J[2]
**percentage of red cell mass in comparison with mean done by HURLEY, P.J[2]

DISCUSSION

In this study, although all the patients had a PCV above 51 which is indicative of erythrocytosis, only three out of seven (42.85%) had an increased red cell mass which confirms that PCV alone is not an accurate indicator of true erythrocytosis. There were great variations between the height, weight, and surface area of the patients included in the study; therefore, consideration should be given to the surface area in the measurement of the red cell mass.

Red cell mass measurement was introduced in Iraq for the first time in 2002; therefore, Polycythemia Vera diagnosis was dependent on PCV and other clinical and laboratory criteria. The results of this study appear to confirm that, red cell mass measurement could make a significant contribution to the differential diagnosis of erythrocytosis.

The primary objective of evaluating erythrocytosis is to ascertain the presence or absence of Polycythemia Vera (PV) and therefore a simple, readily available laboratory test to establish a diagnosis of Polycythemia Vera would be highly desirable; however, none exists[8-10].

The recent discovery of the JAK2 V617F gene mutation may prove helpful in the

diagnosis of Polycythemia Vera[7]. Although this new discovery may change the approach in the diagnosis of Polycythemia Vera, it would be prudent to confirm a diagnosis of erythrocytosis before embarking on doing costly and non available investigations.

This study showed that the increased PCV does not necessarily mean an increased red cell mass but may be only relative erythrocytosis and therefore measurement of red cell mass is necessary to confirm true erythrocytosis.

Insufficient financial investment, the duration of time which was required per case (60 to 90 minutes), availability of equipment and the setting of the study were the principal causes for the small sample size, and therefore a larger sample size would be a strongly recommended for any future study.

CONCLUSION

Red cell mass measurement can be a valuable test to differentiate absolute from relative erythrocytosis as it is still a major criterion in the diagnosis of Polycythemia Vera. PCV alone is not an accurate indication of true erythrocytosis. There were great variations between height, weight and surface area; therefore, surface area should be considered in the measurement.

Further research using larger sample size is required. Multicentric study would be advised for this purpose.

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