

Artigo / Article

## Erythrocyte indices in the second trimester of pregnancy: are reference values well established?

*Índices eritrocitários no segundo trimestre da gravidez: os valores de referência estão bem estabelecidos?*

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*The erythrocyte index values in the pregnancy-puerperal cycle behave in a particular manner; however, the physiological or nosological meaning of such alterations are not well established. This study was aimed at analyzing the distribution of erythrocyte indices in the second trimester of pregnancy. The study was carried out with secondary data from 347 pregnant women in the 2nd trimester of low-risk prenatal care at the Instituto Materno Infantil Professor Fernando Figueira (Recife, Brazil) between May 2000 and June 2001. Measurements were performed using an automatic counter. Frequency distribution curves of erythrocyte indices (means and standard deviations) and correlation curves of hematocrit (HTC) and hemoglobin (Hb) concentrations were constructed. The mean red blood cell number (RBC) was  $3.7 \times 10^{12}$  cells/L; mean HTC was 32.9% and mean Hb was 10.8 g/dL; the values exhibited Gaussian distributions. The mean morphological values were 88.4 fL for the mean corpuscular volume; 29.0 pg for the mean corpuscular hemoglobin and 32.8 g/dL for the mean corpuscular hemoglobin concentration; these values were not adjusted to Normal distributions. HTC values were three times higher than Hb values. Mean RBC, HTC and Hb values (used in the diagnosis of anemia) were lower than thresholds considered normal and exhibited Gaussian distributions. Morphological indices remained within normal ranges, but without delineating a Normal curve. As expected HTC and Hb values behaved following a linear correlation. Rev. Bras. Hematol. Hemoter. 2009; 31(1):37-40.*

**Key words:** Anemia; iron deficiency; prenatal care; erythrocyte indices; pregnancy.

### Introduction

Threshold erythrocyte indices values define anemia and its classifications, but vary with age, gender and physiological conditions such as pregnancy.<sup>1-3</sup> Red blood cells number (RBC), haematocrit (HTC) and hemoglobin concentration (Hb) values indicate the erythroid mass, the

reduction of which results in anemia.<sup>1,2</sup> Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) indicate the morphology (size and degree of hemoglobinization) of the red blood cells, theoretically associated to the cause and functional classification of anemia.<sup>1</sup> The erythrocyte indices behave in a Gaussian manner in the general population.<sup>1</sup>

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The RBC, HTC and Hb values in the pregnancy-puerperal cycle behave following a physiological curve (concavity turned upward), with the lowest point between the 24<sup>th</sup> and 28<sup>th</sup> weeks of pregnancy and a gradual increase from this point until birth, returning to initial levels in approximately the 6<sup>th</sup> week postpartum.<sup>4-8</sup> However, alterations in morphological erythrocyte indices and the nosological meaning of such alterations are not well established. The few studies published<sup>5-9</sup> state that MCH and MCHC can also undergo significant drops in normal pregnancy, becoming accentuated by the end of the third trimester; and that the MCV undergoes an increase, following the same time sequence, however, do not deviate from ranges normal for healthy women at fertile ages and return to pre-pregnancy levels in the late puerperal period.<sup>5,8,9</sup>

Current cutoff points of erythrocyte indices (during pregnancy as well) are derived from a survey carried out between the 1970s and 1990s on the North American population, therefore, likely do not apply to all populations.<sup>3,10,11</sup> Consequently, it is of relevance to describe the behavior of these indices in different subpopulations, particularly during pregnancy, from the presupposition that they will be distributed in distinct manners.<sup>10</sup> The aim of this study was to describe the distribution of RBC, HTC, Hb, MCV, MCH and MCHC in the second trimester of low-risk pregnant women and determine the correlation between haematocrit and hemoglobin concentration values.

**Patients and Method**

This cross-sectional study was based on a secondary databank of a clinical trial.<sup>12</sup> Between May 2000 and June 2001, pregnant women were recruited at the women's prenatal care clinic of the Instituto Materno Infantil Professor Fernando Figueira (IMIP), a tertiary care hospital located at sea level in northeastern Brazil. Subjects who had used medication containing iron in the previous 60 days were excluded. A total of 393 participants with a single, low-risk pregnancy between 16 and 20 weeks were selected. Of these subjects, 347 underwent the hematological examinations analyzed herein.

Erythrograms were performed by a automated electronic cells counter (model Coulter T 890) and included the following variables: RBC in 10<sup>12</sup> cells per liter (10<sup>12</sup> cel/L); Hb in grams of hemoglobin per deciliter of whole blood (g/dL); HTC in percentage of volume of whole blood occupied by the conglomerate of red blood cells (%); MCV in femtoliters (fL); MCH in picograms (pg) of hemoglobin per red blood cell; MCHC in grams of hemoglobin per deciliter of the conglomerate of red blood cells (g/dL).

Statistical analysis was performed using the EPI INFO 6.04 b and Minitab 14.2 software programs, obtaining frequency distribution, mean and standard

deviation. The Anderson-Darling test was used for the compatibility of the variables with Gaussian distribution. Pearson's test was used for the correlation between the HTC and Hb values. The Research Ethics Committee of the Instituto Materno Infantil Professor Fernando Figueira approved the study under n° 650/05.

**Results**

The mass erythrocyte indices (RBC, HTC and Hb) were Gaussians. The same did not occur with the morphological erythrocyte indices (MCV, MCH and MCHC). Due to the large sample size and the proximity of the mean and median values, the option was made to represent them through means and standard deviations (Table 1). Figure 1 illustrates a linear correlation in which each additional unit of Hb corresponded to 2.94 units of HTC, beginning with a virtual minimum value of 1.23.

Table 1. Distribution of erythrocyte indices in 2<sup>nd</sup> trimester of pregnancy. Instituto Materno Infantil Professor Fernando Figueira (IMIP), Recife, Brazil, 2000/2001

Parameter Índice	Average	+/- 2 SD	Normality test*
RBC	3,7x10 <sup>12</sup> /L	3,0x10 <sup>12</sup> - 4,4x10 <sup>12</sup> /L	0,846
HTC	32,9%	27,5 - 38,4%	0,892
Hb	10,8 g/dL	9,0 - 12,6 g/dL	0,293
MCV	88,4 fL	78,6 - 98,2 fL	< 0,005
MCH	29,0 pg	25,1 - 32,8 pg	< 0,005
MCHC	32,8 g/dL	31,0 - 34,5 g/dL	0,032

RBC = red blood cells number; HTC = haematocrit; Hb = hemoglobin concentration; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; SD = standard deviation.

\* Anderson-Darling test

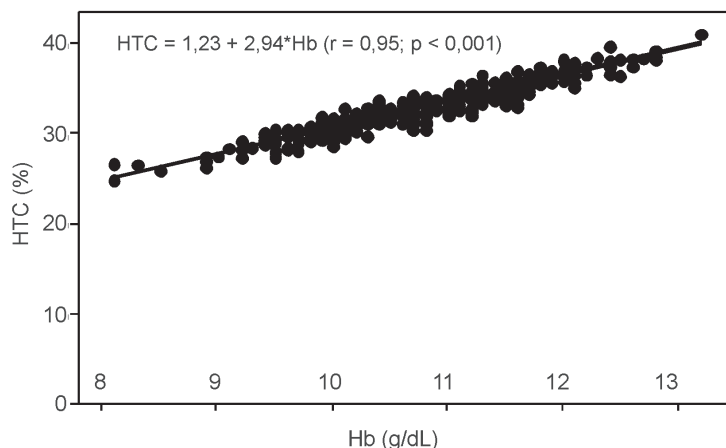


Figure 1. Correlation between hemoglobin concentration (Hb) and haematocrit (HTC) values in 2<sup>nd</sup> trimester of pregnancy. Instituto Materno Infantil Professor Fernando Figueira (IMIP), Recife, Brazil. 2000/2001

Table 2. Studies that describe erythrocyte indices in the 2nd trimester of pregnancy. Medical Literature Analysis and Retrieval System Online (Medline), MDconsult, Scientific Electronic Library Online (SciELO), Literatura Latinoamericana e do Caribe em Ciências da Saúde (Lilacs) and Cochrane (1980-2007)

Authors (year)	Country	Statistic	RBC ( $\cdot 10^{12}$ cél/L)	HTC (%)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Puolakka (1980) <sup>17</sup>	Finland	X ( $\pm 2SD$ )				90 (82-98)		32.7 (29.9-35.5)
Volpi et al. (1991) <sup>18</sup>	Italia	X	4.06		11.5	89.6	28.6	31.9
Knight et al. (1994) <sup>19</sup>	Afroamericanas	X (SD)	3.7 (0.1)	34.3 (0.5)	11.6 (0.1)	89.6 (0.7)	30.6 (0.3)	33.8 (0.3)
Petraglia et al. (1994) <sup>4</sup>	Brazil	X (UL-LL)	3.75 (3.29-4.22)	34.8 (32-37.5)	11.0 (10.0-12.0)			
Van Buul et al. (1995) <sup>8</sup>	Holland	X (p5-p95)	3.8 (3.3-4.4)	34.8 (32-37.5)		89 (82-97)		
Friis et al. (2001) <sup>20</sup>	Africa	X (CI)			11.8 (11.7-11.9)			
Casanova et al. (2005) <sup>16</sup>	Afroamericanas	X (SD)				83.9 (1.0)	28.4 (0.4)	33.8 (1.0)

RBC = red blood cells number; HTC = haematocrit; Hb = hemoglobin concentration; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; X = mean; SD = standard deviation; UL-LL = upper limit - lower limit; p5-p95 = 5percentile - 95percentile. 95% confidence interval (CI).

## Discussion

From the premise of Gaussian distribution<sup>13</sup> regarding the specific case of anemia, 2.5 percent of the subjects would be below the lower threshold of normality for the red blood cells number, haematocrit and hemoglobina levels.<sup>10</sup> The same criteria applies to the size of the red blood cells or their degree of hemoglobinization.<sup>1,2</sup> Hence, in an attempt to reproduce the actual distribution of erythrocyte indices in the present sample, data were analyzed without excluding iron deficiency and not all exhibited the expected Gaussian configuration.<sup>9,14,15</sup> Erythrocyte mass indices (RBC, HTC and Hb) exhibited Normal distribution, whereas morphological indices (MCV, MCH and MCHC) did not follow a Normal curve. This phenomenon may be explained by a possible emergence of young red blood cells of different sizes and degrees of hemoglobinization, recruited by the high erythropoietic demand of pregnancy.<sup>16</sup>

As displayed in Table 2, studies<sup>4,8,16-20</sup> describing erythrocyte indices values in the second trimester of pregnancy generally demonstrate that these values tend not to deviate from ranges considered normal for healthy women at fertile ages.<sup>10</sup> This situation was not corroborated by the present study with regard to RBC, HTC and Hb, as the means of these values were below their respective lower cutoff points. This finding indicates an epidemiologically anemic population, considered to be of a severe magnitude by the World Health Organization ( $\geq 40\%$ ).<sup>10</sup> However, this high frequency could indicate an absolute (true anemia) and/or relative (hemodilution) drop in erythrocyte mass among these pregnant women.<sup>10</sup> Indeed, an analyses of same databank verified that the frequency of anemia whereas the frequency of iron deficiency anemia were 56.6% and 10.7%,

respectively.<sup>21</sup> Therefore, is necessary survey to establish the true erythrocyte indices cutoff points in pregnancy women at the our region.

Milman et al.<sup>5</sup> demonstrated a significant correlation between HTC and Hb throughout pregnancy in patients treated both with and without iron. The resultant function ( $HTC = 0.046 + 2.62 Hb$ ) was similar to that found here, reaffirming a 3:1 correlation empirically observed between the HTC and Hb cutoff points.<sup>10,11</sup> Therefore, there may be incoherence in considering cutoff points that do not obey this rule, as when establishing a 11.0 g/dL threshold for Hb, this would signify a 33% threshold for HTC. Co-linearity could be applied by extension to the RBC and MCV, as these are derived from the HTC; to the MCH, as it is derived from the RBC, and to the MCHC, as it is derived from the Hb/HTC.<sup>1,2</sup>

## Conclusions

In the second trimester of pregnancy, the erythrocyte mass indices routinely used in the diagnosis of anemia were, on average, lower than limits considered normal and reproduced Gaussian distribution. The morphological indices remained within ranges considered normal, but did not delineate a Normal curve. There was the expected co-linearity between HTC and Hb values at a ratio 3:1.

## Resumo

Os valores dos índices eritrocitários comportam-se de maneira particular no ciclo grávido-puerperal, entretanto, o significado fisiológico ou nosológico de tais alterações não está bem estabelecido. Este estudo objetivou analisar as distribuições dos índices

eritrocitários no segundo trimestre da gestação. O estudo foi realizado baseando-se em dados secundários de 347 gestantes de 2º trimestre do pré-natal de baixo risco do Instituto Materno Infantil Prof. Fernando Figueira (Recife - Brasil), entre maio de 2000 e junho de 2001. Foram construídas curvas de distribuição de frequências dos índices eritrocitários, com médias e desvios-padrão, e curva de correlação dos valores de hematócrito (HTC) e de concentração de hemoglobina (Hb), mensurados por contador automático. O valor médio da hematimetria foi  $3,7 \times 10^{12}$  células/L; do HTC, 32,9% e da Hb, 10,8 g/dL, apresentando distribuições gaussianas. As médias foram para volume corpuscular médio de 88,4 fL; hemoglobina corpuscular média de 29,0 pg e concentração de hemoglobina corpuscular média de 32,8 g/dL, não ajustadas à distribuição normal. Os valores de HTC corresponderam a três vezes os de Hb. Hematimetria, HTC e Hb – utilizados para diagnóstico de anemia – foram, em média, inferiores aos limites considerados normais, apresentando distribuições gaussianas. Os índices morfológicos mantiveram-se nas faixas consideradas normais, porém sem delinear curva Normal. O HTC e Hb apresentaram a correlação linear esperada. Rev. Bras. Hematol. Hemoter. 2009; 31(1):37-40.

**Palavras-chave:** Anemia; anemia ferropriva; cuidado pré-natal; índices de eritrócitos; gravidez.

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