Pediatric Metabolic Syndrome and Cell Blood Counts: Bivariate Bayesian Modeling

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Summary

Cell blood counts are components of hematological parameters and indicators of pro-inflammatory states. They are proposed to be associated with metabolic syndrome (MetS). This study aimed to assess the relationship of the white blood cell (WBC) and the red blood cell (RBC) counts with components of MetS in the pediatric age group. The sample consisted of 300 children (152 boys) aged 6–12 years. Hierarchical Bayesian analysis of the bivariate Poisson regression model was used to estimate the effect of various components of MetS according to the cell blood counts. We found that RBC and WBC counts were correlated with the fasting blood glucose, the waist-to-height ratio, serum triglycerides and the blood pressure levels adjusted for age, the body mass index, gender, total cholesterol, low-density lipoprotein cholesterol and the hip circumference. The high-density lipoprotein cholesterol was correlated with the RBC counts based on 95% high posterior density regions for parameters in the Bayesian model. Our findings may serve as confirmatory evidence for the beginning of inflammatory process related to the cardio-metabolic factors from early life.

Key words: Bayesian approach, bivariate count data, cell blood count, childhood obesity, metabolic syndrome.

Introduction

Metabolic syndrome (MetS) is recognized as one of the most serious clustering of risk factors for dyslipidemia and hypertension and is also deliberated as the secondary target for the treatment of coronary heart disease, as well as type 2 diabetes [1, 2]. This syndrome is no more limited to adult populations and to high-income countries, but is also prevalent in the pediatric population of developing countries [3].

Such experience is limited in Asian developing countries. By using the criteria of Adult Treatment Panel-III (ATP-III) adopted for children and adolescents, a study in Turkish children reported a prevalence of 2.2% for MetS [4]. In another study conducted on Korean adolescents, the prevalence of MetS increased significantly from 6.8% in 1998 to 9.2% in 2001 [5]. According to this study, nearly 500,000 out of 5.4 million South Korean adolescents aged 12–19 years were suggested to have MetS.

In a study in China, by using the International Diabetes Federation (IDF) definition, the prevalence of MetS was 0.8% for children aged <10 years. The proportion of children with at least one, two and three components of MetS were 25.0, 5.4 and 0.9%, respectively. Such metabolic abnormalities were also present in children <10 years of age [6].

Results of a study conducted in Iran reported a prevalence of 10.1% in adolescents aged 10–19 years (10.3% in boys and 9.9% in girls), according to modified ATP-III criteria [7].

A nationwide study on Iranian children and adolescents revealed a prevalence of 14.1% for MetS
based on ATP-III criteria; this remarkable prevalence may have resulted from the increasing prevalence of overweight and abdominal obesity among Iranian children and adolescents [8, 9].

Recent studies showed that the prevalence of MetS among overweight/obese children is 10–36% based on the modified ATP-III criteria, 16–44% based on the IDF criteria and 23–42% according to the World Health Organization criteria [10].

Despite differences in overall MetS prevalence estimates in various populations, the current literature consistently indicates that the prevalence of MetS increases with age [11, 12]. These studies also have shown that obesity, high blood pressure, elevated triglycerides (TG) and low levels of high-density lipoprotein cholesterol (HDL-C) are cardiovascular risk factors, which are tracked from childhood to adulthood [13, 14].

Although the exact underlying mechanisms of this important syndrome remain to be determined, many cross-sectional and longitudinal studies showed strong correlation between MetS and some factors based on the inflammation, endothelial dysfunction, oxidative stress, hepatic dysfunction and insulin sensitivity [15–19]. Insulin resistance, considered as an essential core of MetS, is suggested to be associated with hematological parameters, especially with white blood cell (WBC) and red blood cell (RBC) counts. However, limited experience exists on systemic evaluation between various components of MetS and WBC or RBC counts in the pediatric age group [20–25].

Furthermore, as WBC and RBC counts are simply performed in almost all laboratories, they can possibly be used to identify the patients at risk of future cardiovascular diseases.

Current guidelines suggest that although screening for MetS is possibly not necessary in the pediatric population as a whole, its screening is necessary in overweight children [26]. Therefore, screening for the components of MetS in overweight children can simplify the monitoring strategies. It also increases awareness, both at the physician level and in the individual member or families, based on combined risks of disorders.

Ethnic differences are reported for features of MetS in the pediatric population [27]. We, therefore, conducted the present study to investigate the association of important constituents’ hematological parameters with components of MetS among a group of Iranian obese children.

Methods

A total of 300 children, aged 6–12 years, were randomly selected from among obese children referred to the Child Growth and Development Research Center, Isfahan University of Medical Sciences, Isfahan, Iran. The obesity was defined as the age- and sex-specific body mass index (BMI) ≥95 percentiles of the Center for Disease Control and Prevention [28] that are confirmed to be acceptable for Iranian children [29].

Participants were referred from schools, health care centers and public-dependent pediatric clinics of Isfahan University of Medical Sciences. The eligibility criteria included the following: age of 6–12 years; individuals with simple obesity; individuals with a medical history of any chronic disease, e.g. hypertension, genetic syndrome, diabetes, a previous malignancy; individuals with a history of chronic medication use or those receiving medication for acute diseases at least 2 weeks before the study; and the WBC count >15 000 cells/mm³ [30] or the hemoglobin >16 mg/dl [2]. This study was approved by the institutional research and ethics review board at the Faculty of Medicine, Isfahan University of Medical Sciences. The informed written consent was obtained from parents and oral assent from children. The health checkup consisted of physical examination, anthropometric measurements, blood chemistry, including low-density lipoprotein cholesterol (LDL-C), HDL-C, TG, total cholesterol, fasting blood sugar (FBG) and cell blood count including WBC and RBC. The weight was recorded in light clothing to the nearest 0.1 kg on a digital weighing scale, and the height was measured without shoes to the nearest 0.1 cm using a Stadiometer (Seca, Tokyo, Japan). The average of two values for each measurement was used in the data analysis. The BMI was calculated from the weight (kg) divided by the height (m²). The waist circumference (WC) was measured to the nearest 0.1 cm over the skin, midway between the tenth rib and the iliac crest. The hip circumference (HiC) was measured at the widest part of the hip, at the level of the greater trochanter, to the nearest 0.5 cm. A blood sample was collected after a 12-h fast using the standard venipuncture technique by expert nurses. The lipid profile was examined by autoanalyzer (Hitachi, Japan) using standard kits (Pars Azmoun, Iran).

Total WBC and RBC were computed by an automated cell counter (Hitachi, Japan). All measurements were made by the same trained team of general physicians and nurses under the supervision of the same pediatrician, by using calibrated instruments and following standard protocols. Blood samples were taken from the ante-cubital vein between 8:00 and 9:30 a.m. The MetS was defined based on the criteria of the National Cholesterol Education Program (ATP-III) [31] customized for the pediatric population: TG > 100 mg/dl, HDL-C <50mg/dl, the systolic and/or the diastolic blood pressure >95th percentile, the waist-to-height ratio (WHtR) >0.5 [32] and the FBG >100 mg/dl [33].

Statistical analysis

We first explored the frequency distributions of demographic and clinical characteristics. All data
were summarized and displayed as mean ± standard deviation (SD) for the continuous variables.

Independent t-test and Mann–Whitney U test (where assumptions for the t-test could not be met) were used for the comparison of physical and biochemical profiles.

The WBC and the RBC are considered as main outcomes, which we presume to affect each other. So, their relationship needs to be analyzed by a simultaneous equation model.

Fitting Bivariate Poisson regression model is plausible because of considering correlation to investigate the relationship between two important hemato logical parameters (WBC and RBC) and MetS components. In this model fitting, the WBC and the RBC were assumed as dependent variables, while the MetS components as independent variables, adjusting the effect of other covariates, such as age, BMI, gender, cholesterol, LDL and HiC.

In this article, we present a Hierarchical Bayesian approach to report the Bayes estimates and make inferences of model parameter.

We used Markov chain Monte Carlo (MCMC) algorithms, such as the Gibbs sampling, to find Bayes estimates and their corresponding credible intervals of the Bivariate Poisson regression model. The Gibbs sampling approach iteratively generates samples from the full conditional posteriors for any complex model and, unlike the classical methods, makes statistical inferences without imposing large sample size of data sets. Furthermore, in many clinical studies, some measurements are likely to be missing, and thus usual classical estimation methods need to be extended while the MCMC approach imputes automatically missing values and appropriately analyze the data set. In our empirical study, the data set contained many missing values and the sample size was not large enough to use classical methods. Thus, we essentially suggested fitting the underlying complex model by the use of Gibbs sampling approach in the Bayesian framework. After the algorithm was converged, generated samples were used to make Bayesian inferences of model parameters based on their corresponding sample means, sample SDs and 2.5th and 97.5th percentiles, or 95% high posterior density (HPD) regions, of marginal posteriors.

Data were entered and analyzed using Statistical Package for Social Sciences version 20.0 (SPSS, Inc., Chicago, IL, USA) and OpenBugs 3.2.2, a free software for the Bayesian analysis of complex statistical models using Gibbs sampling approach [34].

To start fitting the Bayesian model, we first adopted a class of conditionally conjugate prior distributions, in the sense that both prior and posteriors belong to the same family but with different components. Then, we provided a code in OpenBugs to implement Gibbs sampling (see Appendix).

Results

Summary statistics of several clinical parameters and anthropometric factors for gender are shown in Table 1. Table includes mean ± SD as descriptive statistics on enrollment variables and significant results of the comparison mean analysis according to different gender. Mean trends for both RBC and RBC counts of categorized features of the MetS are listed in Table 2. The complementary results of Table 2 including different categories of gender variables are presented in supplementary file. In general, the obese children with MetS features had higher RBC or WBC counts. Based on statistical test results, there is no statistical difference between WBC and RBC counts in low and high HDL groups (p = 0.44 and 0.29, respectively) and the same for RBC counts, in different groups of TG (p = 0.89).

In this study, the influence of several MetS components on the WBC and the RBC simultaneously were investigated using bivariate Bayesian modeling of count data after controlling potential confounding variables.

Posterior estimates of parameters were obtained using the OpenBugs software. For the underlying model, 10 000 iterations were discarded as burn-in sample to eliminate the impact of starting values and then 100 000 iterations with tuning of 10 followed to obtain Bayes estimates (posterior means and SDs) of parameters. Convergence was assessed by visual assessment of the Markov chain for all parameters. Trace plots of samples and Monte Carlo errors was checked (not shown here). As was suggested for the convergence assessment, ratios of the
Monte Carlo errors relative to the respective SDs of the estimates were <0.05.

Based on 95% HPD, both RBC and WBC counts were significantly correlated with the TG, FBG and HDL-C, but not with the blood pressure levels. WC was correlated only with the WBC count. According to the sign of regression coefficients, there was strong positive correlation between WBC, RBC and FBG ($\beta_{\text{WBC}} = 0.872$, $SD_\beta = 0.0561$; $\beta_{\text{RBC}} = 0.687$, $SD_\beta = 0.0123$). Furthermore, positive correlation existed between the WBC and the RBC counts with TG ($\beta_{\text{WBC}} = 0.0674$, $SD_\beta = 0.002$; $\beta_{\text{RBC}} = 0.0525$, $SD_\beta = 0.001$), BP ($\beta_{\text{WBC}} = 0.0434$, $SD_\beta = 0.011$; $\beta_{\text{RBC}} = 0.068$, $SD_\beta = 0.006$) and WHtR ($\beta_{\text{WBC}} = 0.032$, $SD_\beta = 0.001$; $\beta_{\text{RBC}} = 0.117$, $SD_\beta = 0.001$), respectively. In addition, RBC counts had significant negative correlation with HDL-C ($\beta_{\text{RBC}} = -0.134$, $SD_\beta = 0.009$).

**Discussion**

The MetS is ever more important risk factor in cardiovascular disease. It is found to be associated with improved erythropoiesis, and improved some hematological factors [35–38]. The high prevalence of this syndrome in the presence of multiple risk factors among overweight children could lead to a disproportional increase in cardiovascular disease in adulthood [39].

The related researches proposed that the hyperinsulinemia, one essential element of MetS, probably affects the chronic activation of the immune system and the inflammation [13, 40]. An increasing number of MetS features would be associated with higher degrees of insulin resistance. This finding might show that an increase in the WBC and the RBC counts could be an expression of an insulin-resistant state. According to these findings, we may suggest that an activated cytokine system might contribute to both increased WBC and insulin resistance [41].

Several large population-based samples found that the total WBC counts or the RBC counts were associated with the risk of MetS and its components [42, 43]. For instance, a study revealed that individuals in the highest quartile of WBC or RBC counts received a 3-fold increase in the odds ratio for MetS compared with those in the lowest quartile of WBC or RBC counts [44]. Additionally, other studies reported that WBC counts significantly increased with increasing numbers of MetS components [45]. The current study also showed that the WBC count was positively correlated with the blood pressure, WHtR, TG and FBS, and negatively with the HDL cholesterol. This is similar to the result of some researches that have shown that the WBC count is associated with the TG [46], the blood pressure [21], FBG and WC, as components of the MetS [47]. Some other epidemiological studies noted a relationship between WBC and lipid abnormalities [19, 38]. Illustrating the mechanisms based on the correlation between WBC counts and MetS components, some studies proposed that the insulin resistance probably resulted from a chronic activation of the immune system and the inflammation, especially in obese children [20].

The WBC count is also a documentary marker of cardiovascular disease. It is found to be associated with improved erythropoiesis, and improved some hematological factors [35–38]. The high prevalence of this syndrome in the presence of multiple risk factors among overweight children could lead to a disproportional increase in cardiovascular disease in adulthood [39].

**Table 2**

The means of WBC and RBC counts for different components of MetS

<table>
<thead>
<tr>
<th>Variables</th>
<th>Means of WBC (per mm$^3$)</th>
<th>$p$</th>
<th>Means of RBC (per mm$^3$)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP &lt;120 mm Hg and/or DBP &lt;80 mm Hg</td>
<td>6350 ± 887</td>
<td></td>
<td>4.98 ± 0.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP ≥120 mm Hg and/or DBP ≥80 mm Hg</td>
<td>7368 ± 1900</td>
<td>&lt;0.001</td>
<td>5.22 ± 0.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HFBS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBS ≥100 mg/dl (6.1 mmol/l)</td>
<td>7247 ± 1973</td>
<td>&lt;0.001</td>
<td>5.04 ± 0.44</td>
<td>0.041</td>
</tr>
<tr>
<td>FBS &lt;100 mg/dl (6.1 mmol/l)</td>
<td>8127 ± 2122</td>
<td>&lt;0.001</td>
<td>4.72 ± 0.36</td>
<td>0.0123</td>
</tr>
<tr>
<td>HTG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG ≥100 mg/dl</td>
<td>1982 ± 2121</td>
<td>0.045</td>
<td>5.01 ± 0.33</td>
<td>0.89</td>
</tr>
<tr>
<td>TG &lt;100 mg/dl</td>
<td>7581 ± 1982</td>
<td>0.0561</td>
<td>4.91 ± 0.49</td>
<td></td>
</tr>
<tr>
<td>LHDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL &lt;50 mg/dl</td>
<td>1980 ± 2121</td>
<td>0.001</td>
<td>5.14 ± 0.27</td>
<td>0.003</td>
</tr>
<tr>
<td>HDL ≥50 mg/dl</td>
<td>7269 ± 2185</td>
<td>0.134, SD$\beta$ = 0.687, SD$\beta$ = 0.0123</td>
<td>4.96 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>HWC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHtR ≥0.5 cm</td>
<td>8267 ± 2264</td>
<td>&lt;0.001</td>
<td>4.97 ± 0.44</td>
<td></td>
</tr>
<tr>
<td>WHtR &lt;0.5 cm</td>
<td>7296 ± 2016</td>
<td>0.0525</td>
<td>5.14 ± 0.27</td>
<td></td>
</tr>
</tbody>
</table>

Means of WBC and RBC counts for HBP, high blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HFBS, high fasting blood sugar; HTG, high triglyceride; LHDL, low HDL cholesterol; HWC, high waist circumstance.
Furthermore, a number of researchers have already noted an association between some components of MetS, such as lipid abnormalities and high blood pressure with RBC counts [22, 23]. Based on the relationship between RBC counts and some components of MetS, there is some evidence suggesting that the possibility of hypothesizing the association between RBC counts and MetS might be due to hyperinsulinemia in individuals with MetS components. These studies have addressed that hyperinsulinemia/insulin resistance is correlated with erythropoesis in humans [24]. Another reason for linking RBC counts and MetS components might be due to the fact that an increasing RBC mass may decrease insulin resistance with the high body iron storage and the increased accumulation of iron can interfere with insulin-mediated effects [51].

As there are controversies to determine which mechanism is more plausible and strongly contributed according to association between MetS components and blood cell counts, further surveys are needed.

**Study limitations**

Our study had some limitations. First, the insulin resistance, the key component of MetS, was not measured by laboratory indexes, and thus a direct relationship between insulin sensitivity and RBC or WBC counts was not demonstrated. Second, this study was cross-sectional in design, and thus did not permit the identification of causal relationship between RBC or WBC counts and MetS. Finally, our study population, although with large counts, consisted of participants who regularly underwent physical checkups; it is possible that lifestyle habits, like smoking and psychosocial behaviors, in this population might be different from those in the general population. The main novelty of our research is investigating young children; this may provide information regarding early stages of development of cardio-metabolic risk factors and their relation with the inflammation from early life.

**Conclusion**

We noted that blood count parameters were significantly and evenly associated with some MetS components among young obese children. These findings are in line with studies conducted among adult population, and serve as confirmatory evidence of the association between surrogate markers of the inflammation with cardio-metabolic risk factors from the early life. The suggested pro-inflammatory state in obese children may lead to higher risk of chronic diseases in later life.

**Supplementary Data**

Supplementary data are available at *Journal of Tropical Pediatrics* online.


Appendix

Specification of Bivariate Poisson Regression Model

Let $Y_{ij}$, $i = 1, 2, \ldots, N$, $j = 1, 2$, be blood cell counts of individual $i$ for the outcome $j$ (RBC and WBC). Assume that the $Y_{ij}$, conditioned on the heterogeneity effects $b_{i1}$ and $b_{i2}$, follow Poisson distribution with rate parameter $\lambda_{ij}$ and the correlation of $Y_{i1}$ and $Y_{i2}$ is nonzero. To operationalize, let

$$Y_{ij} | \alpha_j \sim \text{Pois}(\lambda_{ij}),$$

$$\lambda_{ij} = \exp(x_j' \beta_j + b_{ij})$$

where $x_j$ denotes a $k \times 1$ covariate vector and $\beta_j$ is a $k \times 1$ vector of corresponding parameters. The correlation between two response variables $Y_{i1}$ and $Y_{i2}$ is modeled by assuming that unobserved heterogeneity effects $b_{i1}$ and $b_{i2}$ follow bivariate normal distribution $N_2(\mathbf{0}, \Sigma)$, where $\Sigma$ is a covariance matrix. The individual probability density function of counts $Y_{i1}$ and $Y_{i2}$ is given by

$$f(y_{i1}, y_{i2}) = \int \int f_{\text{Pois}}(y_{i1}, y_{i2} | b_{i1}, b_{i2}) f_{\text{Normal}}(b_{i1}, b_{i2}) db_{i1} db_{i2}$$

The marginal log-likelihood function of the above bivariate Poisson-lognormal, is of the form $l(\beta, \Sigma) = \sum \log(f(y_{i1}, y_{i2}))$. This cannot be written in closed form and thus, in a Bayesian framework, the MCMC approaches, such as the Gibbs sampling, may be implemented to make statistical inference of model parameters. The Gibbs sampling implementation using the OpenBugs software is given below.

The OpenBugs Code for Fitting Hierarchical Bayesian Analysis of Bivariate Poisson Regression Model

```openbugs
model{
  for(i in 1:N){
    wbc1[i] ~ dpois(theta.w[i])
    theta.w[i] <- exp(lambda.w[i])
  }
  for(j in 1:N){
    rbc1[i] ~ dpois(theta.r[i])
    theta.r[i] <- exp(lambda.r[i])
  }
  alpha[i,1:2] ~ dmnorm(zero[1:2], Tau[1:2,1:2])
  for(k1 in 1:10){
    beta[k1,k2] ~ dnorm(0, 0.01)
  }
  Tau[1:2,1:2] ~ dwish(R[1:2,1:2], 2)
  Var[1:2,1:2] <- inverse(Tau[1:2,1:2])
}
```