Association between Vitamin D Receptor Gene Polymorphisms with Immune Thrombocytopenic Purpura in Children

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ABSTRACT

Background: Vitamin D receptor (VDR) gene polymorphisms have been demonstrated in the development of autoimmune diseases. Also, vitamin D deficiency has been linked with the development of autoimmune diseases. Immune thrombocytopenia purpura (ITP) is developed secondary to the production of autoantibodies against platelets leading to isolated thrombocytopenia. Objective: This study aimed to evaluate the association between VDR gene polymorphism and susceptibility to ITP in children as well as other prognostic factors. Patients and Methods: A case-control study included 60 subjects and conducted at Hematology Unit of Pediatric Department and Clinical Pathology Department at Zagazig University Hospital. Children were divided into: case group included 30 children with primary ITP and the control group included 30 matched healthy children 15. All the included subjects were subjected to the following: history taking, physical examination, laboratory investigations. Results: There was no statistical significance difference between the studied groups regarding age or sex. Platelets count between the studied groups ranged from 6 to 38 x 10^3/mm^3 with mean 16.63±7.89 and median 17 x 10^3/mm^3. There was no statistical significant relation between VDR BsmI gene polymorphism and sex, frequency of upper respiratory tract infection (URTI), purpura and ecchymosis; but there was a statistical significant increase in frequency of wet bleeding among BB genotype compared to other genotypes. There was no relation between VDR BsmI analysis and line of treatment among the studied cases group. Conclusion: VDR BsmI polymorphism can be used as a risk marker for ITP susceptibility in children. There was an association of VDR BsmI genotype frequency in ITP patients with no effect on platelet count or bleeding severity. Keywords: Thrombocytopenia, Purpura VDR Bsmi, Vitamin D Receptor.

INTRODUCTION

Vitamin D is a steroid hormone precursor that undergoes chemical conversion in the liver and kidney: the first reaction produces 25-hydroxyvitamin D, an objective indicator of vitamin D status, and the second produces the main bioactive form, 1,25-dihydroxyvitamin D (1). Vitamin D receptor polymorphisms are associated with the incidence and severity of certain autoimmune diseases (2). The mechanism by which VDR polymorphism affects autoimmunity is not yet clear, although activation of the receptor contributes to immune responses via regulation of the T-helper (Th)1/Th2 cytokine balance and reduces production of Th2 cytokines (3).

The immune thrombocytopenia purpura (ITP) in children is one of the most common benign hematologic disorders. Isolated, immune-mediated thrombocytopenia is characteristic of it (4). The etiology of thrombocytopenic purpura in the immune system is unclear but is likely due to genetic or acquired factors (5).

Most frequently acquired factors are immunological problems such as cross-reactive antibodies to platelet membrane glycoproteins developed during viral infections such as rubella, varicella, mumps, cytomegalovirus, and Epstein–Barr viruses (6). These viral infections can trigger an autoimmune process leading to a loss of peripheral tolerance, and the production of self-reactive antibodies that destroy platelets. By modulating both innate and adaptive immunity, and regulating the inflammatory cascade, vitamin D has a significant influence on the host's immune system (7).

In recent years 1,25 [OH]2D3 has been rediscovered as an immune modulator. Lassandro et al. (8) suggested that vitamin D deficiency does not represent a chronicity factor for ITP. So, further studies are needed to understand the role of vitamin D in ITP pathogenesis.

The current study aimed to evaluate the association between VDR gene polymorphism and susceptibility to ITP in children as well as other prognostic factors.

PATIENTS AND METHODS

A case-control study included 60 subjected and conducted at Hematology Unit of Pediatric Department and Clinical Pathology Department at Zagazig University Hospital during a period from December 2020 to the end of May 2021.

Inclusion criteria: Children with primary ITP, both sexes, and age between 1 - 15 years.

Exclusion criteria: Children with other causes of thrombocytopenia, children < 1 year and > 15 years, and children with recent manifestations of active infection, or secondary causes of ITP.
Operating design:
The participants were divided equally into: case group (30 children) with primary ITP, and control group (30 children). The diagnosis of ITP was made on the basis of proper assessment of history, with special attention to the history of intake of medications known to cause thrombocytopenia and symptoms suggesting associated viral infections.

All patients were subjected to history of drug intake such as steroids that nearly most of patients conservative pr started by oral steroid at first and shifted to other line regimen of treatment such as intravenous immunoglobulins (IVIG) or started plus IVIG or poor response and history of bleeding (skin, URTI, purpura, ecchymosis, or mucus membrane, frank bleeding).

Laboratory investigations included complete blood count (CBC), reticulocyte count, peripheral blood smears, and direct antiglobulin test, aPTT, bleeding time, and clotting time and stool for H pylori antigen. Bone marrow examination, actually we did not need to do this invasive procedure to our patients as their presentations, clinical scenarios, and laboratory investigations went smoothly for ITP diagnosis.

VDR polymorphisms detection:
VDR BsmI polymorphism (rs1544410) was detected by polymorphism reaction (PCR) followed by restriction fragment length polymorphism analysis (PCR-RFLP). One milliliter peripheral venous blood samples were collected in sterile tubes containing EDTA for DNA extraction. DNA was extracted from whole-blood samples using QIAamp Blood Genomic DNA Kit catalog number: K0781 (USA). For determining DNA concentration, 1 OD unit measured at 260 nm corresponds to 50 μg/ml of DNA. DNA purity was determined by measuring the A260/A280 ratio. For statistical analysis, capital letters represented absence and lowercase letters represented the presence of BsmI restriction site; (B/b). Genotype was determined according to fragments length i.e. homozygote AA (bb) subjects = 822bp product, heterozygote GA (Bb) subjects = 822, 650 and 172bp products and homozygote subjects GG (BB) = 650 and 172bp product. SNP resulting in A-G substitution in VDR gene intron 8 leads to the generation of a BsmI restriction site. Homozygous subjects with alleles containing nucleotide A at this position showed one band at 822 bp and were designated as having bb BsmI genotype. Homozygous subjects with alleles containing G at this position showed 2 bands of 650 and 172bp and were designated as BB. Subjects with heterozygote status showed 3 bands: 825, 650, and 172bp and was designated Bb.

Ethical approval:
The study was approved by the Ethical Committee of Zagazig Faculty of Medicine. An informed consent was obtained from the caregivers of all patients in this research. Every patient received an explanation for the purpose of the study. All given data were used for the current medical research only. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical Analysis
Data were analyzed using SPSS (statistical package for the social sciences) program version 21 for windows and Epi-Info program version for all the analysis. Chi-square $\chi^2$ test, Fischer exact test, Mann-Whitney test, and Kruskal-Wallis test were done. All data were tested with Kolmogorov-Smirnov Z test and so presented with mean ± SD, median and interquartile range (IQR). P value < 0.05 was considered significant.

RESULTS
There was no significant difference between both groups regarding age and gender (Table 1).

Table 1: Demographic data of the studied groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n = 30)</th>
<th>Controls (n=30)</th>
<th>MW</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>8.73±3.02</td>
<td>8.33±3.62</td>
<td>0.42</td>
<td>0.67 NS</td>
</tr>
<tr>
<td>Range</td>
<td>4-14</td>
<td>3-14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>9(6-11)</td>
<td>8.5(5-11.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
<td>15</td>
<td></td>
<td>0.07</td>
</tr>
<tr>
<td>Male</td>
<td>14</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD: Standard deviation IQR: Inter quartile range MW: Mann Whitney test $\chi^2$: Chi square test

The proceeding factor of URTI was found in 46.7%, of our patients (Figure 1).
The most common clinical presentation in our patients was purpura (Table 2).

Table (2): Initial clinical presentation in our patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n=30)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Purpura:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>24</td>
<td>80</td>
</tr>
<tr>
<td>No</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>Ecchymosis:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22</td>
<td>73.3</td>
</tr>
<tr>
<td>No</td>
<td>8</td>
<td>26.7</td>
</tr>
<tr>
<td>Wet bleeding:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td>36.7</td>
</tr>
<tr>
<td>No</td>
<td>19</td>
<td>63.3</td>
</tr>
</tbody>
</table>

Regarding VDR BsmI analysis, the three Cdx-2 genotype distributions in the participants, these results illustrated that in the patient’s, the most presented genotype was the BB genotype. While in the control group the BB genotype was presented (Figure 2).

Table (3): Relation between VDR BsmI analysis and initial platelets count between our patients

<table>
<thead>
<tr>
<th>Gene:</th>
<th>No</th>
<th>Platelets (x10^3/mm^3)</th>
<th>KW</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median</td>
<td>IQR</td>
<td></td>
</tr>
<tr>
<td>bb</td>
<td>6</td>
<td>13.5</td>
<td>10 - 21.5</td>
<td>3.01</td>
</tr>
<tr>
<td>Bb</td>
<td>13</td>
<td>18</td>
<td>12 - 23</td>
<td></td>
</tr>
<tr>
<td>BB</td>
<td>11</td>
<td>10</td>
<td>10 - 19</td>
<td></td>
</tr>
</tbody>
</table>

IQR: Inter quartile range, KW: Kruskal Wallis test

There was no relation between VDR BsmI analysis and 1st line of treatment between our patients (Table 4).
other hand, the study of Hesham et al. (16) reported 100% (20 patients) were given oral steroids and solumedrol, 65% (13 patients) were given IVIG and only 6 patients (30.0%) were given thrombopoietin receptor agonists.

Monticello et al. (17) demonstrated that the level of VDR mRNA was significantly decreased in patients with the VDR B allele versus those not bearing the B allele. To date, a large number of studies regarding the association between BsmI gene polymorphism and autoimmune diseases have been published. Moreover, in case group, BsmI B allele frequency was 58.3% and that for b allele was 41.7%. However, in control group, BsmI B allele frequency was 33.3% and that for b allele was 66.7%. This goes with Abd-Allah et al. (18), El-Barbarya et al. (19) and Elhoseiny et al. (20), but not in agreement with Mansour et al. (21), Emerah and El-Shal (22), Mosaad et al. (23), and Saad et al. (24). This difference might be related the size of each study.

The present study shows that there was no relation between VDR BsmI analysis and line of treatment among the studied cases group. This result goes with the study of Evim et al. (14) and Hesham et al. (16).

CONCLUSION

VDR BsmI polymorphism can be used as a risk marker for ITP susceptibility in children. There was an association of VDR BsmI genotype frequency in ITP patients with no effect on platelet count or bleeding severity.

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Conflict of interest: Nil.

REFERENCES