Human parvovirus B19 in childhood acute lymphoblastic leukaemia in Basrah

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Abstract

Objective: To investigate the association of human parvovirus B19 infection with the onset of acute lymphoblastic leukaemia and its effect on TEL-AML-1 fusion gene and the presence of mutant P53.

Methods: The case-control study was conducted at Basrah Hospital for Paediatrics and Gynaecology, Basrah, Iraq, from May 2009 to April 2010. A total of 100 blood samples were collected from 40 newly diagnosed cases and 60 healthy children to serve as control matched by age and gender. Human parvovirus B19-IgG and anti-P53 antibody were detected by enzyme-linked immunosorbent assay and TEL-AML-1 fusion gene was detected by reverse transcriptase-polymerase chain reaction on extracted ribonucleic acid from fresh blood samples using specified primers. SPSS 15 was used for statistical analysis.

Results: A higher proportion of human parvovirus B19-positive cases was found in leukaemic patients (n=19; 47.5%) compared to 12 (20%) in the control group (p<0.05). There was significant association between TEL-AML-1 translocation and human parvovirus-B19 infection as 10 (71.4%) of TEL-AML-1 translocation-positive cases had human parvovirus-B19 IgG. On the other hand, there was no association between such infections and P53 gene mutation in the patients.

Conclusion: Human parvovirus-B19 infection is common in the population, with higher prevalence among leukaemic patients with significant association between human parvovirus-B19 and TEL-AML-1 fusion gene in patients of acute lymphoblastic leukaemia.

Keywords: Childhood acute lymphoblastic leukaemia, Human parvovirus B19. (JPMA 64: 9; 2014).

Introduction

Oncology patients are at particular risk for human parvovirus B19 (HPV-B19) infection which may cause severe persistent, usually non-specific illness in this group. However, the patient's attenuated immune responses may obscure the serologic and clinical manifestations of infection. Healthy hosts are able to clear the virus within weeks after infection. Persistent HPV-B19 tend to occur in immunocompromised patients and manifest as pure cell aplasia and chronic anaemia. It also causes severe cytopenia and mimics a leukaemic relapse or therapy-induced cytopenia in patients with haematologic malignancies. It is important to consider HPV-B19 infection as a cause of anaemia and suppressed erythropoiesis in children with acute lymphoblastic leukaemia (ALL) receiving ongoing treatment.

Eiji-Moritad et al demonstrated that the B19 non-structural protein (NS-1) mediated G1 arrest is thought to occur via P53 and Rb-independent pathway and the expression of p21/WAF-1 is upregulated in NS-1 transfected cell as well as HPV-B19 infected cells. These results suggest that NS-1 of B19 virus has the ability to promote expression of p21/WAF-1 which should result in G1 arrest. It was also found that there was an enhanced phosphorylation of P53 in HPV-B19 infected cells. The data suggests that P53 plays an important role in B19 virus induced apoptosis via activation of ataxia telangiectasia mutated members (ATM) of the phosphoinoside kinase family by B19-virus infection. ATM is known to directly phosphorylate P53 at serine 15 residue, and the serine 15 phosphorylation of
P53 was upregulated in B19-virus infected cells, suggesting the possibility that ATM kinase is activated by HPV-B19 infection. A pre-leukaemic phase typified by pancytopenia and bone marrow (BM) hypoplasia is well documented prelude to ALL (pre-ALL) in children. HPV-B19 exhibits a marked tropism to human BM and replicates only in erythroid progenitor cells, acting as a confounding but treatable agent in immunocompromised patients. The advent of B19-specific IgG acting in concert with the failing of BM and B19 is possibly one of several factors capable of triggering the onset of pre-ALL as several anecdotal reports described rare cases of ALL diagnosis preceded by a pre-leukaemic phase known as pre-ALL in association with HPV-B19 infections.

This study planned to investigate the association of HPV-B19 infection with the onset of ALL and its effect on TEL-AML-1 fusion gene in the view of P53 protein status.

**Patients and Methods**

The case-control study was carried out from May 2009 to April 2010 on 40 children below 15 years of age, and newly diagnosed, with untreated ALL. The primary diagnosis was based on complete blood picture (CBP) and BM aspirate. The diagnosis of ALL was based on standard French-American-British (FAB) morphologic and cytological criteria by a specialist haematologist and the patients were then referred to the leukaemia treatment unit at Basrah Hospital of Paediatrics and Gynaecology, Basrah, Iraq. An age of 1-9.99 years and white blood cells (WBCs) count less than 50x10⁹/L were considered standard risk criteria, with all other combinations (organomegaly, central nervous system infiltration) being in the high-risk group. Sixty healthy children matched by age and gender, from the general population of Basrah (school children and daycare centres in Abu-Alkhasib and Hartha localities) with negative history of major illnesses, no history of cancer or any apparent congenital anomalies, were recruited as the control group.

Three ml of blood was taken; 1ml in plain tube was allowed to clot and serum was separated into a sterile tube and kept frozen at -20°C until serological examination for the detection of HPV-B19 IgG by enzyme-linked immunosorbant assay (ELISA) (parvovirus-B19-IgG ELISA -DRG international Inc-Germany) and the estimation of anti-P53 by using MESACUP-EIA kit (USA). All procedures were carried out according to the manufacturer's instructions. The cut off value of anti-HPV-B19 IgG was calculated as 0.445 = CO. Patient sera with mean absorbance value more than 20% above the CO was considered positive and sera more than 10% below the CO was considered negative. Optical density (OD) values were converted to DR-unit as described by the kit instructions (positive = >12 DRU; Grey zone = 9-12 DRU; negative <9 DRU; CO= 10 DRU). The level of anti-P53 antibody was determined from the calibration curve which was constructed from the standard calibrator values provided by the manufacturer. Any level >1.3 U/ml indicated the presence of anti-mutant P53. SPSS version15 was used to analyse the data. A 2X2 table was used for data analysis. If one cell of the table contained less than 5 members, Fisher test was used otherwise one had to use Chi-Square test to assess the significance of differences between groups. P value less than 0.05 was considered statistically significant, and P value less than 0.01 was considered highly significant.

**Results**

The cases comprised 20 (50%) males and 20 (50%) females with a mean age of 4.56±2.95 years, while the controls had 31 (51.6%) males and 29 (48.3%) females with a mean age of 4.95±2.62 years. The overall distribution of HPV-B19 IgG antibody among the cases and the controls were noted down (Table-1).
The highest seropositivity was noticed among the cases, 19 (47.5%) compared with 12 (20%) in the controls. The statistical difference was significant (p <0.05).

The relation between TEL-AML-1 translocation and HPV-B19 infection (Table-2) showed that 10 (71.4%) of TEL-AML-1 positive cases were seropositive to HPV-B19 IgG, while 21 (24.4%) of the fusion gene negative cases were seropositive to B19-IgG antibody. This difference was statistically significant (p <0.05).

Besides, 3 (15.8%) of the ALL patients seropositive to HPV-B19 IgG had anti-p53 antibody while none of the healthy controls with HPV-B19 antibody showed positive results to anti-p53 antibody (Table-3).

### Table-1: Distribution of human parvovirus-B19 antibodies in both cases and controls.

<table>
<thead>
<tr>
<th>B19 IgG Ab.</th>
<th>Study population</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study group N(%)</td>
<td>Control group N(%)</td>
</tr>
<tr>
<td>Positive</td>
<td>19(47.5)</td>
<td>12(20)</td>
</tr>
<tr>
<td>Negative</td>
<td>21(52.5)</td>
<td>48(80)</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>60</td>
</tr>
</tbody>
</table>

*Positive: level of B19 IgG > 12 DRU, Negative: <9DRU

\[ \chi^2 = 8.48. \]

\[ df (degree of freedom) = 1. \]

\[ p < 0.05. \]

*The highest seropositivity was noticed among the cases, 19 (47.5%) compared with 12 (20%) in the controls. The statistical difference was significant (p <0.05).*

*The relation between TEL-AML-1 translocation and HPV-B19 infection (Table-2)*

### Table-2: The relation between TEL-AML-1 fusion gene and human parvovirus-B19 antibodies.

<table>
<thead>
<tr>
<th>HPV-B19 IgG N(%)</th>
<th>TEL-AML-1 fusion gene</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive N(%)</td>
<td>Negative N(%)</td>
<td></td>
</tr>
<tr>
<td>Positive (31.0)</td>
<td>10 (71.4)</td>
<td>21 (24.4)</td>
</tr>
<tr>
<td>Negative (69.0)</td>
<td>4 (28.6)</td>
<td>65 (75.6)</td>
</tr>
<tr>
<td>Total</td>
<td>14 (100)</td>
<td>86 (100)</td>
</tr>
</tbody>
</table>
**Table-3: The relation between HPV-B19 seropositivity and anti-P53 antibodies.**

<table>
<thead>
<tr>
<th>Anti-P53 Antibody group</th>
<th>HPV-B19 antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study group</td>
</tr>
<tr>
<td></td>
<td>Positive N(%)</td>
</tr>
<tr>
<td>Positive</td>
<td>3 (15.8)</td>
</tr>
<tr>
<td>Negative</td>
<td>16 (84.2)</td>
</tr>
<tr>
<td>Total</td>
<td>19 (100)</td>
</tr>
</tbody>
</table>

Fisher Exact Test = 1.76.
The difference was statistically not significant (P>0.05).

**Discussion**

Greaves has long postulated that infection plays part in childhood ALL.\(^\text{10}\) Epidemiological (for example population mixing, space time clustering, seasonality, etc) and biological (for example association with particular human leukocyte antigen [HLA] alleles) data suggest a role for infectious agents at some stage in the etiology of childhood ALL. Recent studies have suggested in utero initiation of pre-leukaemic clones\(^\text{11}\) which require one or more subsequent genetic and/or proliferative event(s) to produce leukaemia in ALL during early childhood. However, it is possible that infection may play a role in the initial genetic re-arrangement. There is increasing evidence that its major role lies at the later proliferative stage. The host response to infection may play an additional role in allowing proliferation of the pre-malignant clone.\(^\text{12}\) The host response during B19-associated acute leukaemia may provide insight into possible mechanisms by which the virus may at least precipitate the overt leukaemia.\(^\text{13}\) In this study, there was statistically significant difference in the prevalence of HPV-B19 IgG among ALL cases compared to control group which was 47.5% vs 20% respectively. These results are considered relatively high because all the patients involved in this study were in a high-risk population (leukaemic). HPV-B19 is highly contagious, common and produces mild disease or could be asymptomatic. Zaki et al\(^\text{14}\) found that HPV-B19 IgM demonstrated in 50% of newly diagnosed leukaemia patients. Another study done in Egypt found PV-B19 IgG in 61% of ALL patients.\(^\text{18}\)

However, the exposure to B19 virus in haematological malignant patients was 69.6%.\(^\text{15}\) It appears that there are inherited determinants of symptomatic infection which affect the immune response and possible susceptibility to infection.\(^\text{16}\)

Chromosome translocation to generate the TEL-AML-1 chimeric fusion gene is frequent and early or initiating event in childhood ALL. Our starting hypothesis was that the TEL-AML-1 protein generates and maintains pre-leukaemic clones and the conversion to overt disease requires secondary genetic changes, possibly in the context of abnormal immune responses. These observations suggest a plausible...
mechanism by which dysregulated immune responses to infection might promote the malignant evolution of TEL-AML-1 expressing pre-leukaemic clones. In this study, 71.4% of the TEL-AML-1 positive cases were positive to HPV-B19 IgG. This association was found to be statistically significant. Erythroid suppression and immune cell proliferation are both associated with B19 virus infection and B19 virus has high affinity toward the erythroid progenitor cells and has the ability to induce apoptosis.\textsuperscript{17} So we can consider that B19 together with other co-factors may have a role in the induction of gene translocation and may also be important in the conversion of pre-leukaemic clones to an overt leukaemia.\textsuperscript{17,18} This is in agreement with another study\textsuperscript{19} which reported that TEL-AML-1 fusion transcripts can be detected in lymphoid cell lines after exposure to apoptotic stimuli as pesticides, organic solvents or other chemicals and viruses.\textsuperscript{18,20} The association between HPV-B19 infection and positiveness of gene fusion is of good prognosis to the patient because this fusion can easily break down and return to normal separated genes and may lead to cure of the patient if the causative agents is viral infection, while if the etiology is due to radiation or exposure to chemical carcinogenic agents, this give worse impact on the prognosis, because the fusion is harder and cannot be easily brokendown.\textsuperscript{20} On the other hand, this association may give a hint that HPV-B19 infection in these patients may be considered to be secondary events associated with the transition of silent pre-leukaemic stem cells (pre-LSCs) to overt ALL.\textsuperscript{21}

In the analysed group of patients, no significant association was observed between the presence of anti-P53 antibody and HPV-B19 infection. So we cannot consider the persistence of B19 virus infection as a direct cause of P53 mutation. Many studies showed that P53 gene mutations are associated with increased sensitivity to virus-induced cell death in human leukaemia cell lines because B19 virus has the ability to replicate in and kill transformed and tumour-derived cells in culture\textsuperscript{22,23} and that P53 is dispensable for this process. This is because viral non-structural (NS) protein which is highly conserved among parvoviruses, may be cytotoxic.\textsuperscript{23,24} Several researchers have reported that the intracellular accumulation of autonomous parvovirus NS proteins is cytotoxic, especially in neoplastic cells and that the cytotoxicity of parvovirus NS protein is modulated by cellular factors.\textsuperscript{25}

**Conclusion**

HPV-B19 infection is common among leukaemia patients than in other segments of population. Besides, there is a significant association between HPV-B19 and TEL-AML-1 fusion gene in ALL patients.

**References**