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Serological Evaluation of Major Beta Thalassemia Patients Below15 for Cytomegalovirus Infection in Iran

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Abstract: Thalassemia is one of the most prevalent human genetic diseases. Thalassemia genes are prevalent all over the world especially in countries on Thalassemia belt which Iran is among them. With due attention to the prevalence of this genetic disease in Iran and patients' vital needs for regularly blood transfusion, it is necessary to study Transfusion-transmitted cytomegalovirus infection in this patients. Current study has been carried out on 309 Thalassemia patients below 15 who were receiving blood from therapeutic centers repeatedly. ELISA method was used to determine CMV-IgM seroposivity for cytomegalovirus, Also 225 healthy people below 15 were selected as control group and finally all data were analyzed by SPSS statistical software. The results of this study showed that 12.9 % of under study Thalassemia patients were seropositive for CMV-IgM antibody and 95.1% were negative. Regional separation of patients showed that the patients resided in Tehran are more infected to this virus than patients of other towns. According to this study, prevalence of CMV-IgM antibody significantly higher than normal population, therefore immunity care of these patients and use CMV seronegative blood units and also hematic parents (parents with the same blood and CMV seronegative) are important.

Key words: Thalassemia, cytomegalovirus, CMV-IgM, seropositive, patients, ELISA, SPSS

INTRODUCTION

The thalassemias are the most common monogenic disease in man, they occur at a high gene frequency through out the Mediteteranean, populations, the Middle east, the Indian subcontinent, Mayammar and in a line stretching from Southern China through Tailand and the Malay Peninsula in to the island populations of the pacific. They are seen commonly in countries to which these high frequency populations immigrate.

The Beta-Thalassemia is extremely heterogeneous at the molecular level.

The only form of treatment available for thalassemic children regular blood transfusion, iron chelation therapy attempt to prevent iron overload, judicious use of spelenectomy in cases complicated by hypersplenism and a good standard of general pediatric care, Marrow transplantation has an important role in selected cases (Wetherall and Clegg, 2001; Marshal *et al*., 2006).

Cytomegalovirus (CMV) is an enveloped double-stranded DNA virus that is a member of the Herpesviridae family (Mokarski, 1996). Primary CMV infection is asymptomatic in most patients (Zanghellini *et al*., 1999) however, in certain immunocompromised populations, it can cause serious life-threatening disease. Despite clearing active viral replication, CMV infected individuals remain infected for life with latent CMV, which can reactivate at later times to cause CMV disease (Prosch *et al*., 1999). One way that primary infection can occur is through blood transfusion from donors with active or latent CMV infection (Roback, 2002).

Human Cytomegalovirus (CMV) infects between 40 and 90% o f individuals in various populations (Pass, 2001; Roback, 2003). Transfusion-transmitted CMV (TT-CMV) infection, first described in the 1960s in cardiopulmonary bypass patients, remains a serious infectious complication of blood transfusion. Cytomegalovirus is currently the only human herpesvirus

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that constitutes a significant risk to the safety of the blood supply Transfusion-transmitted CMV is a significant cause of morbidity and mortality, particularly in immunocompromised patients (Alfieri *et al*., 1996; Dollard *et al*., 2005; John *et al*., 2007).

Primary CMV infection of CMV-seronegative patients can occur through transfusion of blood products from CMV-seropositive donors. In seropositive donors, latent CMV is found in peripheral blood monocytes (Slobedman and Mocarski, 1999) to a much lesser extent, CMV also exists as free virus in the plasma of window period donors (Drew *et al*., 2003). After transfusion, latent virus can Presumably reactivate; yielding actively replicating virus that can produce primary infection in the recipient. Although primary CMV infection wills usually only cause serious disease in a small minority of immunocompetent patients, it can pose a significant threat in selected groups of CMV seronegative immunosuppressed patients. These patients can experience complications of CMV infection such as life-threatening pneumonia, retinitis and intestinal disease (John *et al*., 2006).

Prevalence of CMV antibodies in thalassemic patients significantly higher than normal population (Nigro *et al*., 1990; Bronciani *et al*., 1990; Germenis, 1990) also exchange transfusion in neonates can result in prenatal transmission for cytomegalovirus infection in low birth weight neonates (Kothari *et al*., 2006).

Transfusion transmitted cytomegalovirus continues to complicate blood transfusion therapy which can lead to sever morbidity and mortality in immunocompromised patients (Roback *et al*., 2007). Our aim in this study is evolution of repeated blood transfusion role in prevalence of CMV-IgM seroposivity in major beta thalassemia patients below 15.

MATERIALS AND METHODS

In this cross-sectional study, the study population consisted of beta major thalassemia patients who have less than 15 years old and underwent regularly repeated transfusion in Tehran, Sheikh Mofid, Bahrami and Vali Asr pediatric centers in Iran in 2006.

In sum 309 patients and 225 matched normal controls were selected for this study.

The age, sex, height, weight, birth date, birth place were recorded. Serum samples were also obtained for CMV-IgM antibody detection. Sera were stored at -20°C until testing. CMV specific IgM were detected in stored sera using commercial cytomegalovirus antibody test kit (Human), RADIM based upon an enzyme immunoassay method (ELISA) where horse-radish peroxidase is used as an enzyme tracer, the specefity and sensivity of kit was 98.1 and 99.1%, respectively. The collected data from questionnaire and examined sera for CMV- IgM antibody analyzed by SPSS. T test was used for data analysis.

RESULTS

Total 309 major beta thalassemia patients below 15 were tested. One hundred and ffty seven (50.8%) were males and 152 (49.2%) were female. Of the total patients serum samples 40 (12.9%) samples were seropositive for CMV-IgM antibody and 269 (87.1%) samples were seronegative, but in control group serum samples 4.9% of them were seropositive for CMV-IgM antibodies. The prevalence rate of CMV-IgM seroposivity was greater in thalassemia patients than control people. The prevalence rate of CMV-IgM seroposivity regarding to sex shown in Table 1.

Questionnaire data and laboratory examinations result showed that CMV-IgM seroposivity in Tehran resided patients greater than other towns patients, there was significant difference between them (Table 2).

The rate of CMV-IgM seroposivity in patients didn't show significant difference regarding to age grouping ($p>0.05$) this data shown in Table 3.

According to filled questionnaires in this study: 8 (2.5%) of patients were using antiviral drugs; the intervals between transfusions in 12 (6%) of Tehran resided patients and 95 (96%) of other towns patients were 3 weeks, also198 (84%) of Tehran resided patients and4 (4%) of other towns patients have 4 weeks interval period between their transfusions.

Table 1: The prevalence rate of CMV-IgM seroposivity regarding to sex

	Male	Female	Total
CMV-IgM seroposive	20 (12.7%)	20 (13.2%)	40 (12.9%)
CMV-IgM seronegative	137 (87.3%)	132 (86.8%)	269 (87.1%)
Total	157 (100%)	152 (100%)	309 (100%)

Table 2: The prevalence rate of CMV-IgM seroposivity regarding to residence

	Tehran	Other towns	Total
CMV-IgM seroposive	38 (17.9%)	2 (2%)	40 (12.9%)
CMV-IgM seronegative	174 (82.1%)	97 (98%)	271 (87.1%)
Total	210 (100%)	99 (100%)	309 (100%)

Table 3: The prevalence rate of CMV-IgM seroposivity regarding to age grouping

	<5 years	6-10 years	11-15 years
CMV-IgM seroposive	6 (17.6%)	12 (19%)	15 (17.6%)
CMV-IgM seronegative	28 (82.4%)	51 (81%)	70 (82.4%)
Total	34 (100%)	63 (100%)	85 (100%)

DISCUSSION

The first finding of this study was that the prevalence of CMV-IgM seroposivity in Iranian major thalassemic patients is higher than normal people.

Regular blood transfusions for patients with thalassemia have improved their overall survival although these transfusions carry a definite risk of the transmission of certain viruses infection with Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), cytomegalovirus and HIV leads to complication when contribute to the mortality and morbidity of patients with thalassemia (Jamal, 1998).

Both donor and recipient factors influence the risk of TT-CMV infection. Donor factors that can influence the risk of TT-CMV infection include the type of blood product (whole blood vs blood components), the presence of recent infection (viremia) and the age of the blood product. Before 1980, the rates of TT-CMV infection were high (an average of 36%), most likely due to the fact that most transfusions before 1980 were of fresh whole blood (Paloheimo *et al*., 1968; Henle *et al*., 1970; Prince *et al*., 1971). Studies conducted after 1980 that did not use whole blood reported an average incidence of TT-CMV infection of 5.1% (Preiksaitis *et al*., 1985, 1988; Curtsinger *et al*., 1989).

A significantly higher prevalence of CMV antibodies was observed in thalassemic patients. the prevalence among spelectomized patients is higher than non spelectomized thalassemic patients. CMV infection responsible at least in part for immunological disturbances and the susceptibility to other infections in thalassemic patients (Jamal, 1998; Germenis and Politis, 1989).

Although saline-washed RBC was using for the regularly transfusion-dependent patients there are high prevalence of CMV infection (Hwang *et al*., 1990).

Based on studies using patients and clinical specimens, peripheral blood White Blood Cells (WBCs) appear to be the primary vector of TTCMV infections. Transfusion-transmitted CMV is a frequent occurrence when seropositive blood containing normal numbers of WBCs is transfused to at-risk patients (Winston *et al*., 1980; Hersman *et al*., 1982; Meyers *et al*., 1986). The incidence of TT-CMV can be significantly decreased by pre-transfusion leukoreduction, (Bowden *et al*., 1995; Hillyer *et al*., 1994) and CMV does not appear to be transmitted by relatively acellular blood components such as fresh frozen plasma (Bowden and Sayers, 1990). Furthermore, the available evidence suggests that CMV is transmitted primarily as a latent virus, based on the almost uniform absence of replication of competent virus in peripheral blood WBCs from healthy donors (Adler, 1983; Jordan, 1983).

Cytomegalovirus can be present in infected blood as a Cell Free Virus (CFV), cell associated actively replicating virus (CAV) and cell-associated Latent Virus (LV) (Jayarama *et al*., 2006).

In this study was no significant difference for the prevalence of CMV-IgM seroposivity between male and female patients so we think sex hormones don't affect on susceptibility for CMV infection. Also prevalence of CMV-IgM seroposivity in Tehran resided patients was more than other towns resided patients that result of residence place, high dense crowded and blood units heterogeneity.

Interval time between transfusions in other towns resided patients significantly closer than Tehran resided patients that may result of parent think that excessive blood transfusion accelerate treatment of their child.

For leukoreduction we can use several methods such as washing, Spin cool filter,reverse centrifuge and leukotrap filters that last method is most effective.

Recent developments in blood filtration technology allow to production of Leukodepleted (LD) blood products (residual leukocytes $<5\times10^6$ per transfused unit) in the laboratory or at the bedside the potential to prevent adverse effect.

Prestorage filtration may have significant advantage for red cell and platelet production, clinical evidence show that 3log10 leukodepletion prevents or delays febrile reactions, CMV transmission and can reduce the incidence of platelet refractoriness, vCJD transmission, mortality and morbidity and organ dysfunction in patients, but this procedure is relatively expensive and need more evaluation (Norfolk and Williamson, 1995; Yomtovian *et al*., 2001; Ljungman *et al*., 2002; Blajchman, 2006).

Lymphocyte and monocyte subset are removed most effectively by prestorage filtration (Roback *et al*., 2000) and leukoreduction of cellular blood products is an efficient method for preventing CMV infection (Vamvakas, 2005; Narvios *et al*., 2005).

It has been hypothesized that prophylaxis with anti-CMV agents may be used to prevent CMV reactivation in high-risk seropositive individuals, but the use of prophylactic antivirals in seronegative individuals cannot be justified because of the toxicity of these agents, ganciclovir has been associated with myelosuppression and foscarnet can cause renal failure and the fact that most seronegative patients will not develop CMV infection or disease (Stocchi *et al*., 1999). Although CMV immune globulin is less toxic than antivirals, the results of several studies suggest that prophylaxis with CMV immune globulin is not sufficiently effective to recommend for prevention of TT-CMV infection in CMV-seronegative individuals (Kocher *et al*., 2003; Guglielmo *et al*., 1994).

CONCLUSION

In our idea CMV seronegative products should be use for thalassemic patients, furthermore should be use sensitive and specific methods for CMV detection especially for marrow transplantation in seronegative patients.

The inability of serology and filtration to completely prevent TT-CMV infection has motivated efforts to evaluate NAT for CMV DNA as an adjunct prevention method. NAT could potentially decrease the window period between infection and the development of anti-CMV antibodies, reducing the incidence of TT-CMV infection after transfusion of CMV-seronegative units (Preiksaitis, 2003). Alternatively, NAT may allow for the detection of units with residual CMV DNA after filtration (John *et al*., 2006).

CMV-seronegative and leukoreduced blood components are not optimally CMV-safe because exclusive use of these components is still associated with a residual risk of breakthrough TT-CMV infection.

Studies demonstrate that amotosalen and UV-A light inactivates high levels of cell-associated and cell-free CMV in platelet concentrates, thus offering a potentially superior alternative to the selection of CMV-seronegative units and leukocyte reduction filtration as a means to decrease the risk of TT-CMV infection.

Also these patients can select the seronegative donors as a Hematic parent so the collected blood products of this hematic parent will be safe for these patients.

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