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Association between Refractory Thrombocytopenia and the Prevalence of Cytomegalovirus infection

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Abstract:

Background and Objective: Refractory Immune thrombocytopenic purpura is uncommon immunological disorders that is linked to various chronic medical conditions and various viral infections as HCV, HIV and CMV. The study aimed to evaluate the prevalence of CMV infection among patients with refractory ITP. **Patients and Method:** After approval of ethics committee, a total of 30 patients with refractory ITP were evaluated for clinical history, general and regional clinical examination. Patients were evaluated for CBC, serological markers of CMV (IGM and IgG) and bone marrow aspiration cytology. **Results:** The mean age of the studied patients was 34.8 years old with most of the studied patients were females (76.67%). Only one patient was found to have splenomegaly while 10 patients have history of splenectomy. Most of the studied patients didn't have any chronic medical illness while 7 patients were diabetic and 4 patients have hypertension. None of the studied patients were positive for serological markers of HCV, HBV nor HIV. All of the studied patients with refractory ITP were found to have positive CMV IgG while only 1 patient had positive CMV IgM. **Conclusion:** CMV infection is highly prevalent among patients with refractory ITP. IgM for CMV is not recommended for screening of CMV infection among those patients.

Keywords: Thrombocytopenia, viral induced thrombocytopenia, ITP, refractory thrombocytopenia.

1. Introduction

Immune thrombocytopenic purpura (ITP) is an uncommon autoimmune illness with incidence rate of 20/100000 of general population ⁽¹⁾. Refractory ITP is recently defined as bleeding with platelet count less than 20000/ μ l of blood with no or short response to corticosteroids or intravenous immunoglobulin ⁽²⁾. It is usually presented by mild symptoms as petechiae and purpura but could progress to severe sequences as intracranial hemorrhage or gastrointestinal bleeding. ITP is usually induced by chronic diseases as systemic lupus erythematosus (SLE), chronic lymphocytic leukemia (CLL) or viral infections as hepatitis C virus (HCV), immunodeficiency virus (HIV) and Cytomegalovirus ⁽³⁾.

Clinical and laboratory reassessment of patients with resistant ITP is essential to rule out alternative causes thrombocytopenia. ITP is mainly identified by ruling out other causes of thrombocytopenia. Diagnosis of ITP is strongly supported by presence of low platelet count, normal bone marrow aspiration, and a response to corticosteroids and IVIG ⁽⁴⁾.

Cytomegalovirus (CMV) infection is common causes of morbidity and mortality in immunocompromised patients while in immunocompetent patients it is typically presented as a symptomatic infection or as mononucleosis-like illness ⁽⁵⁾.

The association between CMV infection and ITP have been described in many ways. CMV is one of common inducing agents that could lead to ITP. One of the proposed links between CMV and ITP is immune-mediated death of infected cells or impairment of bone marrow stromal function, or CMV-induced direct cytotoxicity to hematopoietic cells ⁽⁶⁾. In the current study we will assess the incidence of CMV among patients with refractory immune thrombocytopenic purpura.

2. Patients and methods

After approval of ethics committee of Faculty of Medicine, Suez Canal University, the present descriptive cross sectional study was conducted among a total of 30 patients diagnosed with refractory immune thrombocytopenic purpura presented to the hematology outpatient clinic and inpatient ward of internal medicine department at Suez Canal University Hospitals. Patients suffering from myelodysplasia, aplastic anemia or bone marrow failure, hematological and or non-hematological malignancies, pregnant females with pregnancy induced thrombocytopenia, patients with organ-specific failure, patients with auto-immune diseases (SLE, RA or multiple sclerosis, etc.) and patients on drugs as aspirin, heparin, quinidine and anticonvulsants were excluded from the study.

Methods of the study:

An informed written consent was obtained from all patients. Patients were evaluated via clinical history assessment, general and regional clinical examination with special emphasis on cutaneous manifestations or bleeding per any orifices.

Laboratory assessment: All patients were assessed for Complete blood count, serological assessment of CMV-specific antibodies IgM and IgG by antibody capture enzyme-linked immunosorbent assay (ELISA) using commercial kits of the types ET1-C1TOK-M and ETI-CITOK-G, respectively (Sorin Biomedica, Italy).

Bone marrow aspirate had been carried out to all patients in the study at Suez Canal University hospital with the aid of clinical pathology department according to the standard hematological techniques described by Dacie and Lewis practical haematology ⁽⁷⁾. Bone aspirate was obtained from sternal body puncture.

Sternal puncture technique:

While the patient is lying in supine position, the angle of the sternum is determined by gentle digital palpation. To find the point where the mid-sternal line and the sternal angle connect, use the needle cover. The patient is instructed to extend his neck in order to make the procedure easier. After disinfecting the region, a surgical aperture drape is placed over it. To anesthetize the skin and subcutaneous tissue, 3 milliliters of 1% lidocaine are used as a local anesthetic. Next, 1 milliliter of anesthetic is subcutaneously injected with lidocaine at several locations along the cortical bone. Following a three-to-five-minute delay, the process begins. The bone marrow needle's components are first identified. Typically, hematopathologists use needles in sizes 16 and 18. At the previously designated location, the needle will be placed through the skin and subcutaneous tissue and up against the bone. After that, the needle is inserted into the bony cortex and rotated on its axis to become anchored at the required depth. After that, the stylet is removed, a 20 ml syringe is attached, and a cautious aspirate is made. Just one milliliter of fluid is sucked for a smear sample.

Statistical analysis:

Gathered information was processed using SPSS version 25 (SPSS Inc., chiago, IL, USA.). Quantitative data was expressed as means $\pm$ SD while qualitative data was expressed as number and percentages (%). A probability value (p-value) <0.05 was considered statistically significant.

3. Results

The mean age of the studied patients was 34.8 years old with most of the studied patients were females (76.67%). Only one patient was found to have splenomegaly while 10 patients have history of splenectomy. Most of the studied patients didn't have any chronic medical illness while 7 patients were diabetic and 4 patients have hypertension. None of the studied patients were positive for serological markers of HCV, HBV nor HIV (**Table 1**).

As presented in table 2, all of the studied patients with refractory ITP were found to have positive CMV IgG while only 1 patient had positive CMV IgM (**Table 2**).

Table 1: Studied Patient clinical characteristics:

Gender	Males	7 (23.33%)
	Females	23 (76.67%)
Age	Mean $\pm$ SD	34.8 $\pm$ 14.69
Clinical characteristics	Splenomegaly	1 (3.3%)
	Splenectomy	10 (33.3%)
Chronic medical illness	No	23 (76.7%)
	Hypertension	4 (13.3%)
	Diabetes Mellitus	7 (23.3%)
Viral markers	HCV Ab	0 (0%)
	HBsAg	0 (0%)

	HIV	0 (0%)
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Table 2: CMV serological markers prevalence among the studied patients:

CMV markers	Number	Percentage
CMV IgM	1	3.3%
CMV IgG	30	100%

Discussion

A complete absence of response to one or more single-agent treatments (such as rituximab and TPO-RA) was the definition of refractory ITP in 2020. Not long after, Miltiadous et al. designated the term "refractory" to individuals whose platelet counts are extremely low and accompanied by bleeding, who do not respond to at least two treatments, and who do not respond to a single medicine. It is not a given that these patients who are refractory necessarily have had splenectomy ⁽⁸⁾.

It has been documented that CMV can both initiate and maintain ITP ^(9,10). The global herpesvirus known as cytomegalovirus (CMV) is quite common all over the world. With a prevalence of almost 100% in Africa, 80% in Europe, and 80% in North America, it is a widespread virus ⁽¹¹⁾.

According to a study by **Gawad et al.**, ⁽¹²⁾ Egypt has a high CMV infection rate. According to Gawad et al., the study revealed a significant CMV seroprevalence among Egyptian blood donors. Out of 88 examined blood samples, 96.6% of blood donors in their study tested positive for CMV ⁽¹²⁾.

A Cross-sectional study was conducted among a total of 30 patients suffering refractory immune thrombocytopenic purpura. Immunologic assessment of cytomegalovirus infection by viral antibodies IgM and IgG using ELISA technique had been done to patients in both groups.

The current study aimed to determine the prevalence of CMV infection among patients with refractory of ITP. We have found that all of the studied patients with refractory ITP were found to have positive CMV IgG while only 1 patient had positive CMV IgM.

Previous study didn't reveal significant association between CMV infection and thrombocytopenia. **Levy and Bussel** ⁽¹³⁾ found that the resolution of thrombocytopenia did not appear to be correlated with CMV clearance. They came to the conclusion that it doesn't appear likely that significant thrombocytopenia is frequently caused by CMV infection. Therefore, it does not appear warranted to test for CMV infection on a regular basis in patients with otherwise typical ITP. Nonetheless, there is still a chance to screen very resistant persons, and it can be useful to investigate the immune systems of CMV-infected individuals ⁽¹³⁾.

A recent Egyptian study by **Mokhtar and colleagues** ⁽¹⁴⁾ has concluded that among Egyptian patients with chronic ITP, CMV appeared to be highly prevalent and they found that the IgM component of the CMV serological assay was not a reliable measure of viral infection. This is consistent with the current findings ⁽¹⁴⁾.

A previous Chinese study have found that 19% of the ITP patients had an IHC-positivity rate for CMV. This suggests that CMV may be a major cause of ITP and that all ITP-affected people should have their CMV markers looked into ⁽¹⁵⁾.

We concluded that CMV infection is highly prevalent among patients with refractory ITP. IgM for CMV is not recommended for screening of CMV infection among those patients. However, the current study didn't confirm the association between CMV and refractory ITP and regular screening of those patients for CMV infection can't be recommended.

This study helped to give an overview of the prevalence of viral antibodies IgM and IgG to cytomegalovirus among patients with refractory ITP patients. More detailed study with comparison to other types of ITP and newly diagnosed patients is required to further emphasize the role of CMV infection in ITP patients.

Conflicts of interest.

None of the researchers has any conflict of interests.

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