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# Cytomegalovirus infection among Patients with Naive versus Refractory Thrombocytopenia

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## ABSTRACT

**Background:** Refractory Immune thrombocytopenic purpura is uncommon immunological disorders that is linked to various chronic medical conditions and various viral infections as HCV, HIV and Cytomegalovirus (CMV).

**Objective:** This investigation aimed to evaluate the occurrence of CMV infection among cases with refractory Immune Thrombocytopenic Purpura (ITP). **Patients and Method:** A total of 30 patients with refractory ITP and another group of 31 patients with naïve 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:** Both groups showed no statistically significant difference regarding age, sex and chronic medical disorders. None of the studied patients were positive for serological markers of HCV, HBV nor HIV. All of the studied patients with refractory or naïve ITP were found to have positive CMV IgG.

**Conclusion:** CMV infection is highly prevalent among patients with ITP with difference between refractory and naïve ITP. No link could be established between CMV infection and refractory ITP.

**Keywords:** Thrombocytopenia, Viral-induced thrombocytopenia, ITP, Refractory thrombocytopenia.

## INTRODUCTION

The total lack of response to one or more single agent treatment was the definition of refractory Immune thrombocytopenic purpura (ITP). The term refractory ITP was introduced to patients whose platelet counts are extremely low and accompanied by hemorrhage, and do not respond to at least two treatments with or without splenectomy [(1)]. It is recently defined as bleeding with platelet count less than 20000/µl of blood with no or short response to corticosteroids or intravenous immunoglobulin [(2)].

The clinical presentation of ITP is usually mild including symptoms as petechiae and purpura. Severe cases could progress to intracranial hemorrhage or gastrointestinal bleeding with serious complications and possible fatal outcome. Despite the fact that ITP is usually regarded as a benign condition, the refractory subgroup of patients' experiences considerable mortality, morbidity, and quality of life impacts as a result of both the disease and its treatment [(3)].

Acquired thrombocytopenia usually results from circulating auto-anti-platelet antibodies that are supposed to attack megakaryocytes and/or restrict platelet formation in bone marrow in addition to attacking and killing the platelets peripherally [(4)].

Various chronic diseases and viral infections has been linked to ITP [(3)]. It was previously described that CMV infection can both elicit and maintain ITP [(5, 6)]. CMV has a high prevalence worldwide reaching up to 100% in some African regions [(7)].

The association between CMV infection and ITP have been described in many ways. CMV is one of frequent 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 [(8)]. In the current study we will assess the prevalence of CMV among patients with refractory immune thrombocytopenic purpura in comparison with patients with naïve ITP.

## PATIENTS AND METHODS

Following the ethics committee's permission, the current descriptive cross-sectional investigation was carried out among two groups of cases with ITP at Suez Canal University's Faculty of Medicine. A total of thirty-one cases with naive ITP comprised the first group, while thirty cases diagnosed with refractory immune thrombocytopenic purpura comprised the second group. All cases have been recruited from the Internal Medicine Department's Inpatient ward and Hematology Outpatient Clinic at Suez Canal University Hospitals.

**Exclusion criteria:** 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 have been excluded from the investigation.

## METHODS OF THE STUDY

Cases 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 IgG and IgM utilizing antibody capture enzyme-linked immunosorbent assay (ELISA) utilizing commercial kits of the types ET1-C1TOK-M



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and ETI-CITOK-G respectively (Sorin Biomedica, Italy).

**Bone marrow aspirate:** It 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 **(9).** Bone marrow aspirate was obtained from sternal body puncture.

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

**Ethical considerations: All research procedures have been authorized by The Ethics Committee of the Internal Medicine Department of the Faculty of Medicine at Suez Canal University. The administrative consents that were necessary have been obtained. The objective of this investigation was to conduct human research in accordance with the Declaration of Helsinki, the ethical code of the World Medical Association.**

***Statistical analysis***

SPSS version 25 (SPSS Inc., Chicago, IL, the states) has been used to analyze the collected data. The means ± SD were used to convey quantitative data, while qualitative data has been expressed as percentages (%) and numbers. The Chi square test has been utilized to evaluate the significance of the distinction among the two groups in terms of qualitative parameters, while the t test was utilized for quantitative variables. A probability value (p-value) of $\leq 0.05$ has been considered statistically significant.

## RESULTS

The mean age of the studied patients was 34.8 years old with most of the studied patients were females (76.67%) among patients with refractory ITP versus mean age of 38.06 years old with 67.74% of the naïve ITP patients were females without statistically significant difference. Only one patient with refractory ITP was found to have splenomegaly versus 4 of patients with naïve ITP with p-value > 0.05. None of naïve ITP patients had history of splenectomy versus 10 patients of refractory ITP patients. Most of the studied patients didn't have any chronic medical illness. None of the studied patients were positive for serological markers of HCV, HBV nor HIV among refractory ITP patients while only 2 patients with naïve ITP were positive for HCV antibodies (Table 1).

**Table (1):** Studied patient clinical characteristics

| | | Naïve ITP (n=31) | Refractory ITP (n=30) | p-value |
|---|---|---|---|---|
| Gender | Males | 10 (32.26%) | 7 (23.33%) | 0.4 (NS) |
| | Females | 21 (67.74%) | 23 (76.67%) | |
| Age | Mean ± SD | 38.06 ± 15.38 | 34.8 ± 14.69 | 0.5 (NS) |
| Clinical characteristics | Splenomegaly | 4 (12.9%) | 1 (3.3%) | 0.1 (NS) |
| | Splenectomy | 0 (0%) | 10 (33.3%) | 0.001* |
| Chronic medical illness | No | 29 (93.5%) | 23 (76.7%) | 0.1 (NS) |
| | Hypertension | 1 (3.2%) | 4 (13.3%) | |
| | Diabetes Mellitus | 2 (6.5%) | 7 (23.3%) | |
| Viral markers | HCV Ab | 2 (6.5%) | 0 (0%) | 0.8 (NS) |
| | HBsAg | 0 (0%) | 0 (0%) | - |
| | HIV | 0 (0%) | 0 (0%) | - |

NS: no statistically significant variance *Statistically significant variance.

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 with also all of the naïve ITP patients were positive for CMV IgG (Table 2).

**Table (2):** CMV serological markers prevalence among the studied patients

| CMV markers | Naïve ITP (n=31) | Refractory ITP (n=30) | p-value |
|---|---|---|---|
| CMV IgM | 0 (0%) | 1 (3.3%) | 0.3 (NS) |
| CMV IgG | 31 (100%) | 30 (100%) | - |

NS: no statistically significant difference.

## DISCUSSION

It has been documented that CMV can both initiate and maintain ITP [5, 6]. The global herpesvirus known as cytomegalovirus (CMV) is quite common all over the world. With a prevalence of almost 100% in Africa, eighty percent in Europe, and 80% in North America, it is a widespread virus [7, 10].

According to a study by **Gawad *et al.*** [11], Egypt has a high CMV infection rate. Also, 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 [11].

The current study was designated with the goal to determine the prevalence of CMV infection among patients with refractory ITP in comparison with patients with naïve ITP. There was statistically insignificant variance among refractory and naïve ITP patients with regard to the presence of CMV infection.

Similarly, previous study didn't reveal significant association between CMV infection and thrombocytopenia. **Levy and Bussel** [12] 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 [12].

A recent Egyptian study by **Mokhtar and colleagues** [13] 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 wasn't a reliable measure of viral infection. This is consistent with the current findings [13].

A previous Chinese study have found that 19% of the ITP patients had an IHC-positivity rate for CMV. This recommends that CMV might be a major cause of ITP and that all ITP-affected people should have their CMV markers looked into [14].

Unlike the current findings, various reports have shown that treating the underlying CMV infection might help treat thrombocytopenia. CMV-associated ITP has been linked to severe thrombocytopenia that is resistant to normal treatments. However, testing for CMV as part of the diagnostic workup is still not recommended [15].

In cases where there is a strong clinical suspicion of CMV exposure, steroid-dependent ITP, or prior to splenectomy, we think it is reasonable to screen for CMV infection. As a result, it could be necessary to modify the ITP guidelines from the American Society of Hematology as well as those from other organizations. The guidelines for testing of CMV throughout pregnancy and before splenectomy should be part of such changes, as treating CMV may enhance ITP and remove the need for invasive operation. Furthermore, this might promote payment for CMV testing in cases with isolated ITP, who do not exhibit any other CMV infection symptoms or signs, and raise clinician knowledge of CMV-induced ITP.

## CONCLUSION

We concluded that CMV infection highly occurred among cases with ITP whether naïve or ITP with no statistically significant difference. IgM for CMV is not recommended for screening of CMV infection among those patients. The current study didn't show evidence supporting linking the CMV to refractory ITP. This study helped to give an overview of the occurrence of viral antibodies IgG and IgM to cytomegalovirus among patients with refractory ITP patients. More detailed studies to compare to other types of ITP and newly diagnosed patients is required to further emphasize the role of CMV infection in ITP patients.

## DECLARATIONS

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**Competing interests**: None.

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