Viral Hepatitis B and C Infection in Patients with Idiopathic Thrombocytopenic Purpura Treated with Triple Therapy

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Abstract:

Background: The immune thrombocytopenic purpura (ITP) disorder is linked to antiplatelet antibodies that destroy platelets. The most crucial factor in diagnosing it is a platelet count of less than 100 x 10^9/L. The purpose of this study was to determine the prevalence of viral HB and C infection in ITP patients treated with triple therapy versus those treated only with steroids. Additionally, the study aimed to examine the impact of viral HB and C infection on the clinical picture, treatment response, and side effects in ITP patients treated with steroids or triple therapy.

Methods: This prospective longitudinal study was conducted on 100 patients with clinical and laboratory evidence of ITP eligible for triple or steroid therapy between December 2019 and June 2022. Patients were divided into two equal groups: Group A: ITP received triple therapy, and Group B: ITP received steroid therapy. Quantitative PCR was planned only for cases with positive serological markers.

Results: After 6 months of therapy, patients treated with triple therapy had significantly higher platelets count than those who received steroids only. Also, random blood sugar was significantly lower in the steroid group. All groups had negative serology regarding HCV Abs and HBsAg during follow-up. Post-treatment platelet count had an insignificant correlation with all baseline variables.

Conclusions: For patients with ITP, triple therapy is a safe, well-tolerated, and successful treatment.

Keywords: Viral Hepatitis B, Viral Hepatitis C, Idiopathic Thrombocytopenic Purpura, Triple Therapy.

Introduction:

Three percent of people worldwide are afflicted with blood-borne viral hepatitis, specifically B and C viruses. In Egypt, the estimated prevalence of hepatitis B and C infection was 6.3% and 1%, respectively, in 2015. The Egyptian Ministry of Health has initiated a nationwide screening and treatment program to eradicate hepatitis C infection by 2020. The program began in October 2018. (1)

In the context of a widespread purpuric rash, immune thrombocytopenic purpura (ITP) is defined by the American Society of Hematology as isolated thrombocytopenia (platelet count < 100,000/microL) with normal white blood cells and normal hemoglobin. ITP was once referred to as ITP or idiopathic thrombocytopenic purpura. Primary ITP is defined as ITP with no underlying condition or secondary etiology; secondary ITP is defined as ITP with an underlying cause or problem. This might

include drug-induced or systemic illness-induced ITP (e.g., SLE, HIV, COVID, etc.). (2)

Rituximab, cyclosporine, and dexamethasone are the combos that were recently authorized for ITP. Hence, it has been demonstrated that suppressing all three immune cell types is more efficient at keeping the pathogenic loop intact. People may risk potentially fatal infections if these cells are suppressed concurrently. And the awakening of ones that have been latent, such as viral infections of hepatitis B and C.

Twenty percent of individuals with ITP have serological evidence of hepatitis C virus (HCV), which is arguably the most common chronic viral infection. However, in patients with freshly diagnosed HCV, the incidence of ITP is higher than expected. There is no known cause for HCV-associated thrombocytopenia; however, it is most likely complicated. Platelet GPIIb/IIIa is cross-reactive with antiviral antibodies associated with ITP-HCV. (3)

Even in the absence of thrombocytopenia, antiplatelet glycoprotein antibodies are frequently seen. Nowadays, it is widely recognized that medications such as anticancer treatments and glucocorticoids might impair the host immune system's capacity to inhibit HBV replication. HBsAg-positive individuals and those with serological evidence of a previously treated HBV infection may experience viral reactivation (HBVr). (4)

Egypt has made significant progress in preventing the spread of hepatitis B and C, primarily due to the President of Egypt's initiative to eradicate hepatitis C in Egypt by 2020. We believe that now is the right moment to identify high-risk individuals who have the potential to spread illness across the community.

This is the first study to evaluate viral hepatitis B and C infection in ITP patients receiving triple treatment domestically and abroad. Studies on patients receiving chemotherapy for hematological malignancies, however, demonstrated the reactivation of latent hepatitis B infection and the seroconversion of HBsAg-negative to positive.

This study aimed to determine the prevalence of viral HB and C infection in ITP patients receiving triple therapy vs a group receiving only steroid treatment. This study aimed to determine the prevalence of viral HB and C infection in ITP patients receiving triple therapy vs a group receiving only steroid treatment. And to investigate the influence of viral HB & C infection on the clinical picture, response to treatment, and side effects in ITP patients who received triple therapy or steroids.

Patients and Methods:**IRB: 17101088 A prospective longitudinal research was conducted**

100 patients with clinical and laboratory evidence of ITP and eligible for triple or steroid therapy between December 2019 and June 2022. The study was done following approval from the Assiut University Hospitals Faculty of Medicine on clinicaltrials.gov (NCT04113915). Informed written consent was obtained from the patients.

Exclusion criteria were age < 18, thrombocytopenia other than ITP, pregnancy, and ITP patients on other treatment modalities.

Patients were divided into two equal groups: Group A, ITP received triple therapy. Group B, ITP received steroid therapy.

Every patient underwent: taking a history (age, sex, duration, previous therapy, and its duration). Clinical data and presentation were recorded. At baseline and before therapy initiation, laboratory investigations are performed (Complete blood picture, liver function tests, coagulation profile, kidney function test, hepatitis markers, HCV antibody, and hepatitis B surface antigen). Quantitative

PCR was planned only for cases with positive serological markers.

Treatment of the Study Patients

Group A: Rituximab 100 mg (ampule) weekly (from 4 to 6 weeks) for days 7, 14,21, and 28, Sandimmune 3-5 miq per kg per day from day 1 to day 28 with continuous follow-up of trough level of Sandimmune, pulse dexamethasone 40 mg (5 ampules) for the first 4 days of the start of triple therapy. **Group B:** Pulse dexamethasone 40 mg (5 ampules) in the first 4 days, then shift to oral steroids by 1 mg per kg daily for 2 weeks, according to the follow-up CBC as platelet count still above (50 ×103/μL). There is no bleeding; we decrease the steroid by 5 mg weekly (gradual withdrawal).

The main indicator of response was the platelet count. Follow up at the outpatient clinics at 15-day intervals in the first month, then monthly till 6 months, and report any clinical insult such as orificial bleeding, eruptions, or side effects. After six months of therapy, a full clinical and laboratory reassessment was performed.

Sample Size Calculation:

The sample size was determined using Open Epi (ver. 3) and Egypt's 6.3% HCV prevalence and 95% confidence interval. To account for dropouts and refusals, 10% was added to the minimum needed sample of 90 individuals. For the trial, a total of 100 participants were enrolled.

Statistical Analysis

The statistical software for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA), was used to analyze the recorded data. The mean ± Standard Deviation (SD) was used to express quantitative data, and the Student t-test was used to compare them. The Chi2 test was used to compare the frequency and proportion of qualitative data. Since there was a 95% confidence level, a P value of less than 0.05 was considered significant.

Results:

Demographic, Clinical, and Laboratory Data of the Study Patients

There were insignificant differences between the groups regarding age, body mass index, and clinical and laboratory data. The majority of patients were females and came from rural areas. Ten patients of the triple therapy group and 17 (34%) patients of group B were smokers. The most frequent manifestations among the studied groups were ecchymosis, epistaxis, and bleeding per the gums, followed by hematuria and hematemesis. Five patients in each group present with melena. Both groups had negative serology regarding HCV Abs and HBsAg, as shown in Table 1.

Table 1: Demographic, clinical, and laboratory data of enrolled patients (n = 100)

		group A (n=50)	group B (n=50)	P value
Age (years)		46.98 ± 12.34	47.98 ± 11.1	0.11
Sex	**Male**	18 (36%)	23 (46%)	0.20
	Female	32 (64%)	27 (54%)	
Residence	**Rural**	36 (72%)	32 (64%)	0.26
	Urban	14 (28%)	18 (36%)	
Body mass index (kg/m^2)		23.49 ± 1.78	24.04 ± 1.61	0.81
Smoking		10 (20%)	17 (34%)	0.17
Occupation	**Farmer**	10 (20%)	7 (14%)	0.17
	Housewife	35 (70%)	26 (52%)	
	Employee	5 (10%)	7 (14%)	

		group A (n=50)	group B (n=50)	P value
clinical data	**Ecchymosis**	40 (80%)	41 (82%)	0.50
	Epistaxis	39 (78%)	42 (84%)	0.30
	Bleeding per gum	40 (80%)	38 (76%)	0.40
	Hematuria	16 (32%)	19 (38%)	0.33
	Hematemesis	4 (8%)	6 (12%)	0.37
	Melena	5 (10%)	5 (10%)	0.63
Laboratory investigations	**Leucocytes (10^3/ul)**	5.48 ± 1.31	6.01 ± 1.45	0.19
	Neutrophils (10^3/ul)	3.56 ± 1.14	4.26 ± 1.82	0.22
	Lymphocyte (10^3/ul)	1.25 ± 0.75	1.81 ± 0.61	0.30
	RBCs (10^3/ul)	4.68 ± 0.85	4.78 ± 0.64	0.08
	Hemoglobin (g/dl)	10.69 ± 2.22	10.15 ± 2.65	0.07
	Hematocrit value (%)	39.13 ± 7.01	37.40 ± 8.03	0.42
	Platelets (10^3/ul)	56.79 ± 6.26	54.14 ± 10.34	0.27
	MPV (fl)	10.27 ± 1.93	10.34 ± 1.64	0.16
	INR	1.10 ± 0.21	1.07 ± 0.73	0.08
	Urea (mmol/l)	9.94 ± 7.48	16.94 ± 9.65	0.23
	Creatinine (mmol/l)	101.94 ± 24.80	108.25 ± 18.87	0.18
	Albumin (mg/dl)	35.10 ± 5.73	35.97 ± 5.33	0.27
	Bilirubin (umol/l)	9.20 ± 3.45	9.82 ± 2.83	0.56
	Direct bilirubin (umol/l)	3.79 ± 1.22	4.98 ± 1.09	0.08
	AST (u/L)	45.96 ± 9.45	40.23 ± 32.23	0.17
	ALT (u/L)	44.09 ± 11.34	54.83 ± 8.98	0.20
	ALP (u/L)	101.77 ± 63.44	114.45 ± 83.87	0.81
	RBS (mg/dl)	190.56 ± 26.78	191.34 ± 30.34	0.13
ABO system **A positive** **B positive** **AB positive** **O positive** **A negative**		 25 (50%) 10 (20%) 8 (16%) 5 (10%) 2 (4%)	 23 (46%) 9 (18%) 11 (22%) 4 (8%) 3 (6%)	0.19
HCV Ab		0	0	---
HBsAg		0	0	---

Data are presented as mean ± SD or frequency (%). BMI: Body mass index

Follow-up Laboratory Data of the Study Patients

There were insignificant differences regarding follow-up laboratory data between the groups, except for significantly higher mean platelet count in group A. Meanwhile, the random blood sugar was significantly lower in group B. Both groups had negative serology as regards HCV Abs and HBsAg during follow-up, as depicted in **Table 2**

Table 2: Follow-up laboratory data of the study

	Group A (n=50)	Group B (n=50)	P value
Leucocytes (10^3/ul)	6.45 ± 1.21	6.70 ± 1.22	0.19
Neutrophils (10^3/ul)	5.01 ± 1.11	5.11 ± 1.80	0.22
Lymphocyte (10^3/ul)	0.98 ± 0.80	1.01 ± 0.55	0.30
RBCs (10^3/ul)	4.50 ± 0.81	4.90 ± 0.62	0.08
Hemoglobin (g/dl)	12.01 ± 2.11	11.99 ± 2.56	0.07
Hematocrit value (%)	41.45 ± 4.44	41.11 ± 8.11	0.42
Platelets (10^3/ul)	178.98 ± 12.12	145.67 ± 20.20	**< 0.001**
MPV (fl)	10.11 ± 1.22	10.21 ± 1.32	0.16
RBS (mg/dl)	245.67 ± 55.56	190.45 ± 45.32	**< 0.001**
HCV Ab	0	0	---
HBsAg	0	0	---

Correlation of Post-treatment Platelet Count with Baseline Laboratory Data of the Study Patients

Post-treatment platelet count had an insignificant correlation with all baseline variables in groups A and B, as shown in **Table 3**.

Table 3: Correlation of post-treatment platelet count with baseline data in the current study

	r value	P value
Age (years)	0.12	0.34
Body mass index (kg/m^2)	-0.23	0.31
Leucocytes (10^3/ul)	-0.09	0.21
Neutrophils (10^3/ul)	0.19	0.23
Lymphocyte (10^3/ul)	-0.23	0.98
RBCs (10^3/ul)	-0.09	0.98
Hemoglobin (g/dl)	0.01	0.34
Hematocrit value (%)	0.04	0.54
Platelets (10^3/ul)	0.22	0.10
MPV (fl)	0.21	0.46
INR	0.25	0.19
Urea (mmol/l)	0.09	0.47
Creatinine (mmol/l)	-0.15	0.78
Albumin (mg/dl)	-0.03	0.39
Bilirubin (umol/l)	0.20	0.18
Direct bilirubin (umol/l)	-0.19	0.70
AST (u/L)	0.11	0.08
ALT (u/L)	0.25	0.19
ALP (u/L)	0.08	0.16
RBS (mg/dl)	0.12	0.11

Discussion:

Platelet destruction by the reticuloendothelial system is the primary cause of decreased platelet counts, which characterize ITP, an acquired autoimmune illness. Primary ITP is the diagnosis when no further thrombocytopenia-related conditions or causes exist. (5)

In the current study, no case was detected to have viral hepatitis B or C either before or 6 months after triple therapy. Triple therapy was more effective than steroid therapy, with a significantly higher platelet count at 6 months after therapy, and random blood sugar was significantly lower in the steroid group. There are various distinctions between HBV infection, HCV infection, and immunosuppression. The differences in the viral architecture, replication mechanisms, and natural infection histories might cause this. (6)

Although cytotoxic T lymphocytes attacking infected cells are widely known to be the cause of HBV and Hepatocytolysis related to HCV, liver disease tends to exacerbate and advance more quickly in individuals with weakened immune systems. This is not the result of the host response healing after treatment termination; it occurs when the immune system is inhibited. This was further demonstrated by the fact that end-stage chronic liver disease (CLD) often develops in a range of years in both healthy individuals and various immunocompromised patients. (7)

In the current study, no single case of viral hepatitis was detected either before or after treatment with triple therapy. No previous study discussed the frequency of HCV and HBV after triple therapy in patients with ITP. However, people receiving treatment for hematological malignancies have been known to reactivate their viral hepatitis B infection (7). Additionally, much research has been done on the connection between viral hepatitis C infection and ITP. Numerous investigations have found a close association with various reasons (8). To treat primary immune thrombocytopenia (ITP), promising findings of combined immunosuppression using high-dose dexamethasone and rituximab have recently surfaced (9) (10). Hey points to the possibility of increasing treatment effectiveness. An earlier investigation was carried out to characterize the safety, effectiveness, and tolerability of intravenous low-dose rituximab 100 mg on days 7, 14, 21, and 28, oral cyclosporine 2.5 to 3 mg/kg daily on days 1 through 28, and oral dexamethasone 40 mg on days 1 through 4. According to the authors ' findings, the 6-month response rate was 60%, no significant unfavorable side effects were connected to therapy, and the treatment was well tolerated. At 12 and 24 months, the responders had respective relapse-free survival rates of 92% and 76%(9).

Additionally, according to a recent study, triple treatment (TT4) produced a strong response. It was well tolerated, with a substantial rise in the mean platelet count after the first, second, third, and fourth weeks compared to the baseline. In the sixth month, 75% of patients (30 out of 40) had a mean platelet count of less than 30 X 109/L. At 12 and 24 months, the treatment-free survivals (TFS) were 93.3% (28/30 patients) and 80% (24/30 patients), respectively. Especially in a setting with limited resources, TT4 T is a successful therapeutic choice that keeps the platelet count at the appropriate level and produces a better-sustained response (10). Numerous additional trials found that chronic ITP patients' platelet counts rose when they received weekly doses of rituximab 375 mg/m2 and dexamethasone for four days at a decreased risk of adverse effects. This may offer a new treatment alternative. This combination has been linked to a higher

platelet count when compared to dexamethasone monotherapy. (11), (12),(13)

Based on our findings, patients with persistent ITP could benefit from 100 mg of rituximab once a week for four weeks. Compared to Yan et al.'s (13) trial, which combined high-dose dexamethasone and low-dose rituximab 100 mg weekly without cyclosporine A in patients who were steroid-dependent or failed to respond to treatment, our study demonstrated the synergistic benefit of cyclosporine A. Although the exact processes by which cyclosporin A (CSA) cures intractable ITP remain unclear, immunological tolerance failure is the primary cause of autoimmune disorders since autoreactive T and B cells identify self-antigens and trigger a cellular/humoral immune response. Thus, it's possible that the inhibitory actions of rituximab and CSA on T and B cells successfully suppressed ITP. (14)

Refractory ITP is difficult to treat and may not have the best results. Our management technique includes observation, consideration, diagnosis confirmation, and a tiered treatment plan for individuals needing therapy. A higher percentage of patients than ever before can maintain hemostatic platelet counts with acceptable tolerability and safety, thanks to recent advancements in treatment, such as rituximab and the TRAs, regarding RBS and elevated BP. Still, current therapeutic options fail for a subset of highly refractory patients. For these patients, new treatments are surely needed. Novel agents, including an anti-CD40 ligand (BMS-986004) and a splenic tyrosine kinase inhibitor (fostamatinib), as well as a number of repurposed drugs (e.g., decitabine, oseltamivir, sirolimus, and thalidomide), are currently in clinical trials.

The main limitations of the current study included being conducted in a single center, a relatively small sample size, and having a short-term duration of follow-up. Yet, the main point of strength in this study was that

it was the first study that discussed the comparison of triple therapy and steroids therapy in patients with ITP. In addition, the frequency of hepatitis viral infection among those patients should be assessed. Although the detected rate of viral hepatitis infection was 0%, this may be secondary to the small sample size of this study. Moreover, it could signify the success of the preventive measures for viral hepatitis infection in Egypt. In addition, the results indicated good control of blood-borne viral hepatitis in our center.

Conclusions:

Triple therapy is a safe, well-tolerated, and effective therapy for patients with ITP.

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Conflict of Interest:
The authors declare that they have no competing interests.

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