بسم الله الرحمن الرحيم

∞∞∞∞

تم رفع هذه الرسالة بواسطة /صفاء محمود عبد الشافى

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

ملاحظات: لايوجد

Evaluation of the Effect of Therapy on the Expression of Platelet Collagen Receptor Glycoprotein VI (GPVI) in Pediatric Patients with Chronic and Persistent Immune Thrombocytopenia

Thesis

Submitted for Partial Fulfilment of M.D. Degree in Pediatrics

By

Michael Botros Elkes Morcos Morcos

M.B.B.Ch (2012), M.Sc (2017)

Under Supervision of

Prof. Azza Abd El Gawad Tantawy

Professor of Pediatrics

Faculty of Medicine – Ain Shams University

Prof. Nayera Hazaa El Sherif

Professor of Pediatrics

Faculty of Medicine – Ain Shams University

Prof. Fatma Soliman Elsayed Ebeid

Professor of Pediatrics

Faculty of Medicine – Ain Shams University

Dr. Eman Abdel Rahman Ismail

Consultant of Clinical Pathology

Faculty of Medicine – Ain Shams University

Dr. Mahmoud Adel Kenny

Assistant Professor of Pediatrics

Faculty of Medicine – Ain Shams University

Faculty of Medicine - Ain Shams University

2022

Acknowledgment

First and foremost, I feel always indebted to GOD, the Most Kind and Most Merciful.

I'd like to express my respectful thanks and profound gratitude to ***Prof. Azza Abd El Gawad Tantawy,*** *Professor of Pediatrics - Faculty of Medicine- Ain Shams University for her keen guidance, kind supervision, valuable advice and continuous encouragement, which made possible the completion of this work.*

I am also delighted to express my deepest gratitude and thanks to ***Prof. Nayera Hazaa El Sherif,*** *Professor of Pediatrics, Faculty of Medicine, Ain Shams University, for her kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.*

I am deeply thankful to ***Prof. Fatma Soliman El-Sayed Ebeid,*** *Professor of Pediatrics, Faculty of Medicine, Ain Shams University, for her great help, active participation and guidance.*

I wish to introduce my deep respect and thanks to ***Dr. Eman Abdel Rahman Ismail,*** *Consultant of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her kindness, supervision and cooperation in this work.*

I would like to express my thanks to ***Dr. Mahmoud Adel Kenny,*** *Ass. Professor of Pediatrics, Faculty of Medicine, Ain Shams University, for his support till this work was completed.*

Last but not least my sincere thanks and appreciation to all patients participated in this study.

Michael Botros Elkes Morcos Morcos

ABSTRACT

Background: GPVI is the predominant platelet receptor for collagen that is essential for platelet adhesion and aggregation. Eltrombopag has been reported as an effective treatment for thrombocytopenia in chronic immune thrombocytopenia (ITP), yet its effect on platelet function in are not fully characterized.

Aim: This prospective trial aimed to investigate the efficacy and safety of Eltrombopag therapy in pediatric patients with persistent and chronic ITP and its effect on platelet adhesion through assessment of glycoprotein VI (GPVI) receptor expression and soluble GPVI levels.

Methods: Persistent or chronic ITP patients aged 1-18 years were screened, 36 were enrolled and divided equally into 2 groups either to receive eltrombopag therapy or the standard of care. All patients were followed-up for 12 months with assessment of bleeding score &complete blood count (CBC). Evaluation of the quality of life, bone marrow reticulin stain, GPVI expression using flow cytometry and measurement of the soluble form of GPVI by enzyme linked immunosorbent assay (ELISA) at baseline and at 6 months were performed.

Results: Among all the studied patients, there were significant negative correlations between bleeding score and each of GPVI expression by flowcytometry and soluble GPVI levels and a positive correlation between platelet count and soluble GPVI levels. ITP patients who were on eltrombopag had significantly lower bleeding score after 6 months of therapy while the quality of life has significantly improved. Platelet count was significantly increased throughout the study. Moreover, GPVI expression by flowcytometry and soluble GPVI level were significantly increased after therapy. Similar results were found when compared with those not on eltrombopag. The number patients who lost complete response (CR) or response (R) was significantly lower among eltrombopag group than those without. Further follow-up of ITP patients who were on eltrombopag till 12 months after therapy showed that they were able to maintain a good quality of life and low bleeding score throughout the period.

Conclusions: Our data suggest that Eltrombopag through its role in up-regulation of glycoprotein VI (GPVI) receptor expression and increase soluble GPVI levels, might have an effect on the platelet functions therefore reduce the bleeding manifestations, decrease the bleeding score and improve the quality of life of chronic and persistent ITP children independent of it effect on platelets count.

Keywords: Platelet Collagen Receptor Glycoprotein VI; Immune Thrombocytopenia

List of Contents

List of Tables

List of Tables (Cont...)

List of Tables (Cont...)

List of Figures

List of Figures (Cont...)

List of Figures (Cont...)

List of Abbreviations

Abb.	Full term
ADCC	*Antibody-dependent cellular cytotoxicity*
ADP	*Adenosine diphosphate*
ALPS	*Autoimmune lymphoproliferative syndrome*
ANCA	*Antineutrophil cytoplasmic antibodies*
ANCOVA	*Analysis of covariance*
CBC	*Complete blood count*
CONSORT	*Consolidated Standards of Reporting Trials*
CR	*Complete response*
CRH-2	*Cytokine receptor homology domain*
CTCAE	*Common Terminology Criteria for Adverse Events*
CVID	*Common variable immune deficiency*
ELISA	*Enzyme linked immunosorbent assay*
FcR	*Fc receptor*
FITC	*Fluorescein isothiocyanate*
GP	*Glycoprotein*
GPVI	*Glycoprotein VI*
IBLS	*ITP Bleeding Scale*
IBLS	*ITP Bleeding Scale*
Ig	*Immunoglobulin*
ITP	*Immune Thrombocytopenia*
MFI	*Median fluorescence intensity*
MKs	*Megakaryocytes*
MSC	*Mesenchymal stem cells*

List of Abbreviations (Cont...)

Abb.	Full term
OPSIs	*Overwhelming post-splenectomy infections"*
PE	*Phycoerythrin*
PE	*Physical examination*
PETIT2	*Pediatric Patients with Thrombocytopenia from Idiopathic Thrombocytopenic Purpura*
sGPVI	*Soluble GPVI*
SLE	*Systemic lupus erythematosus*
SoC	*Standard of Care*
TPO	*Thrombopoietin*
TPO-R	*TPO receptor*
TPO-RA	*Thrombopoietin receptor agonist*
TRAs	*TPO receptor agonists*
Treg	*T-regulatory*
WHO	*World Health Organization*

ABSTRACT

Background: GPVI is the predominant platelet receptor for collagen that is essential for platelet adhesion and aggregation. Eltrombopag has been reported as an effective treatment for thrombocytopenia in chronic immune thrombocytopenia (ITP), yet its effect on platelet function in are not fully characterized.

Aim: This prospective trial aimed to investigate the efficacy and safety of Eltrombopag therapy in pediatric patients with persistent and chronic ITP and its effect on platelet adhesion through assessment of glycoprotein VI (GPVI) receptor expression and soluble GPVI levels.

Methods: Persistent or chronic ITP patients aged 1-18 years were screened, 36 were enrolled and divided equally into 2 groups either to receive eltrombopag therapy or the standard of care. All patients were followed-up for 12 months with assessment of bleeding score &complete blood count (CBC). Evaluation of the quality of life, bone marrow reticulin stain, GPVI expression using flow cytometry and measurement of the soluble form of GPVI by enzyme linked immunosorbent assay (ELISA) at baseline and at 6 months were performed.

Results: Among all the studied patients, there were significant negative correlations between bleeding score and each of GPVI expression by flowcytometry and soluble GPVI levels and a positive correlation between platelet count and soluble GPVI levels. ITP patients who were on eltrombopag had significantly lower bleeding score after 6 months of therapy while the quality of life has significantly improved. Platelet count was significantly increased throughout the study. Moreover, GPVI expression by flowcytometry and soluble GPVI level were significantly increased after therapy. Similar results were found when compared with those not on eltrombopag. The number patients who lost complete response (CR) or response (R) was significantly lower among eltrombopag group than those without. Further follow-up of ITP patients who were on eltrombopag till 12 months after therapy showed that they were able to maintain a good quality of life and low bleeding score throughout the period.

Conclusions: Our data suggest that Eltrombopag through its role in up-regulation of glycoprotein VI (GPVI) receptor expression and increase soluble GPVI levels, might have an effect on the platelet functions therefore reduce the bleeding manifestations, decrease the bleeding score and improve the quality of life of chronic and persistent ITP children independent of it effect on platelets count.

INTRODUCTION

Novel thrombopoiesis-stimulating agents have been developed increasing platelet production and thereby correcting the thrombocytopenia in ITP patients ***(Cines et al., 2002).*** These compounds called TPO receptor agonists (TRAs) show no structural resemblance to Thrombopoietin (TPO) but still bind and activate the TPO receptor (TPO-R) ***(Meletis et al., 2010).***

Patients with ITP often have few bleeding symptoms despite very low platelet counts, suggesting that platelets are highly functional ***(Karpatkin, 1969; Panzer et al., 2007).*** Although TPO stimulation in vitro does not directly activate platelets, it potentiates platelet reactivity to several agonists, including adenosine diphosphate (ADP), thrombin, and collagen ***(Harker, 1998).*** However, the effect of eltrombopag on platelet function in vivo in thrombocytopenic patients is still debatable and requires extended studies because of its potential for thrombosis with normal platelet counts. The hyposthesis of its effect on platelet activation is suggested through either direct stimulation of platelet TPO receptors rendering them more susceptible to lower concentrations of agonists or indirectly by the influx of new potentially more reactive platelets ***(Psaila et al., 2012).***

Glycoprotein PIbα of the GPIb-IX-V complex and GPVI are of particular interest because these receptors are essentially