

# Relationship Plasma levels of Von Willbrand Factor with peripheral blasts and chemotherapy in Syrian Children with Acute Lymphoblastic Leukemia

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## ABSTRACT

The danger for thromboembolic disorder is raised in long-term stayers of infant acute lymphoblastic leukemia (ALL) because of endothelial malfunction (ED). Adverse endothelial malfunction occurs through the acute stage of disease. The levels of von Willbrand factor antigen (VWF:Ag) increase in neoplastic diseases and appear from harmful changes in the endothelium. Our purpose of this study is to verify plasma levels of (VWF:Ag) in children who diagnosed ALL recently, and compare them with peripheral blast cells (PBs) and chemotherapy. Our study involved thirty patients with acute lymphoblastic leukemia (17 males and 13 females) with their ages from 2 to 12 years. VWF:Ag levels were analyzed by enzyme linked immunosorbent assay (ELIZA) and measured at the start of diagnosis (D0) and at day (D35) of chemotherapy for 30 ALL children with and without peripheral blast cells (PBs). Our results indicated that patients with PBs had higher median plasma VWF:Ag concentrations than individuals without ( $99.69 \pm 72$  ng/ml vs  $64.18 \pm 40.14$  ng/ml,  $P < 0.001$ ). Following induction therapy, children with peripheral blasts showed increase in VWF:Ag concentrations, whereas children didn't have peripheral blasts had no significant variation in VWF:Ag concentrations ( $64.18 \pm 40.14$  ng/ml vs.  $49.30 \pm 28.4$  ;  $P = 0.652$ ). In conclusion, significantly raised plasma levels of VWF in children with acute lymphoblastic leukemia may be appeared through acute stage and remission of the disease approve the injury to the endothelial lining of blood vessels and cause endothelial malfunction in childhood ALL.

**KEY WORDS:** Von Will brand Factor, Peripheral Blasts, Acute Lymphoblastic Leukemia.

## 1. INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant condition developing as of the clonal multiplying of lymphoid precursors including stopped maturation (Zuckerman and Rowe 2014). It is the most popular cancer in children, acts for almost one-third of all childhood cancers with a peak occurrence in children aged 2–5 years (Hagag, Abdel-Lateef, 2014). Despite the high rate of healing, ALL is one of the leading reasons of mortality in pediatric (Nigro 2013). The danger for cardiovascular disease which may caused by endothelial malfunction (ED) is increased in long - term stayers of infant acute lymphoblastic leukemia (ALL) (Jarvela, Niinikoski, 2013). A limited studies demonstrate that endothelial malfunction is part of ALL and may be seen in ALL children at the diagnosis (Doroszko, Niedzielska, 2016). In addition, Chemotherapy used in ALL protocols might cause direct endothelial injury, possibly rising the of endothelial malfunction and hence leads to progress of late cardiovascular events in ALL stayers (Jarvela, Niinikoski, 2013). The vascular endothelium is concerned in the manufacture of many important elements which are implicated in cardiovascular pathophysiology. One element which is produced by, and stored in, endothelial cells is Von Will brand factor (VWF) (Lip and Blann 1997). It is a multimeric glycoprotein existing in blood plasma, and synthesized in both endothelial cells and megakaryocytes. It facilitates platelet connection and accumulation in the positions of vascular injury. VWF also bounds to coagulation factor VIII in the circulation, stabilizing the later in plasma. It performs a significant function in hemostasis: its paucity causes Von Willbrand disease (VWD), the most familiar congenital hemorrhage condition in humans (Starke, Ferraro, 2011), and increased levels of VWF are may be a valuable indirect marker of thrombosis (Spiel, Gilbert, 2008). VWF manufactured in endothelium is either put in storage that called Weibel Palade bodies (WPBs) or constitutively secreted into the plasma, which ensures circulating VWF concentrations (Casonato, Cattini, 2016). Many studies point out that plasma levels of VWF should reveal endothelial function, and aberrant concentrations would indicate endothelial malfunction and injury (El Sherif, Narouz, 2014). The assay of such a beneficial indicator of endothelial malfunction may be a non-invasive method of evaluation the diagnosis or as a pointer of disease development (Lip and Blann 1997). Our study targeted to measure VWF:Ag concentrations in ALL children and their association with peripheral blast (PB) cells and chemotherapy and investigate the prognostic significance of this marker.

## 2- MATERIALS AND METHODS

**Study groups:** our prospective study involved thirty children (17 males and 13 females) with their ages from 2 to 12 years and newly diagnosed with ALL at Children University Hospital in Damascus. Classification and diagnosis of ALL were depended on the French–American–British (FAB) criteria and Immunophenotyping analyses. ALL children were divided into two groups: 24 ALL children with characterized PB and 6 ALL children without PB. Peripheral blast cells were determined by a hematologist. All children enrolled on BFM90 protocol. Induction

therapy in this protocol included: 60 mg/m<sup>2</sup> prednisolone (PRD) for 4 weeks, 1.5 mg/m<sup>2</sup> VCR, 30mg/m<sup>2</sup> DNR and 5.000 iu/m<sup>2</sup> ASP. Patients were asked for past events of coagulation or bleeding tendency. Informed written parental consent was given.

Blood samples were gathered at the start of diagnosis (D0) and during the first remission at day (D35) for 30 ALL children recently diagnosed. A sample of 5mL of intravenous blood was obtained from patients in the fasted state and collected into tubes preloaded with sodium citrate. The sample was directly centrifuged at 1500g for 15 minutes. Plasma VWF:Ag was measured using enzyme linked immunosorbent assay (ELISA) kit manufactured by SunRed company, China.

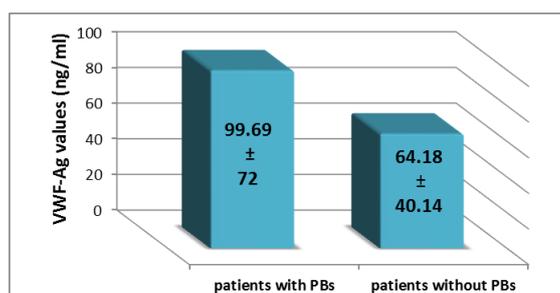
**Statistics:** data were evaluated utilizing SPSS (version 21, IBM SPSS) and Microsoft Excel 2013. Values were expressed by mean  $\pm$  SD. Mann-Whitney U-test was applied to associate between the means of two groups. We considered  $P < 0.05$  as a statistically significant.

### 3. RESULTS

The mean of VWF:Ag plasma concentrations was significantly higher in All patients with PBs ( $99.69 \pm 72$  ng/ml) at diagnosis than patients without PBs ( $64.18 \pm 40.14$  ng/ml), ( $p=0.032$ ).

**Table.1. VWF plasma concentrations in ALL patients with and without PBs at the start of diagnosis (D0)**

	N	VWF:Ag (ng/ml)
Patients with peripheral blasts	24	$99.69 \pm 72$
Patients without peripheral blasts	6	$64.18 \pm 40.14$
p-value		$<0.05$

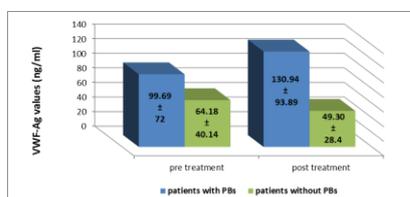


**Figure.1. VWF plasma concentrations in ALL patients with and without PBs at diagnosis**

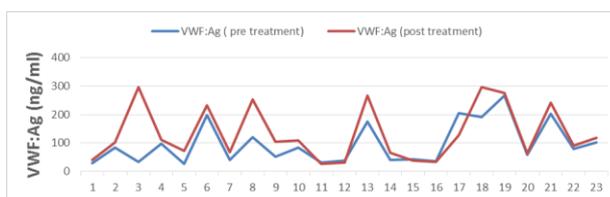
After treatment, VWF:Ag plasma concentrations were significantly higher in All patients with PBs ( $130.94 \pm 93.89$  ng/ml) at day 35 of chemotherapy than patients without PBs ( $49.30 \pm 28.45$ ), ( $p=0.043$ ).

**Table.2. VWF plasma concentrations in ALL patients with and without PBs at day 35**

	N	VWF:Ag (ng/ml)
Patients With peripheral blasts	24	$130.94 \pm 93.89$
Patients Without peripheral blasts	6	$49.30 \pm 28.45$
p-value		$<0.05$



**Figure.2. VWF plasma concentrations in ALL patients with and without PBs at day 35.**



**Figure.3. VWF plasma concentrations in ALL patients with and without PBs pre and post chemotherapy.**

### DISCUSSION

Our study showed at diagnosis that children who had blast cells in peripheral blood had significantly higher levels of VWF:Ag than individuals didn't have blast cells ( $p < 0.001$ ).

We elucidated this elevation in VWF levels by several reasons. First, that circulating blasts may selectively motivate production and release of VWF antigen through endothelial stimulation. The endothelial stimulation and VWF:Ag then lead to elevation in parameters of thrombin generation causing the stimulus of prothrombotic state seen in ALL patients (El Sherif, Narouz, 2014). Second, the patients with blasts cells could have increased inflammatory response (Athale, Moghrabi, 2010), induced, by the disease or infections. Inflammatory response brakes the antithrombotic state and enhances the prothrombotic state of endothelial cells, and hence elevates concentrations of VWF:Ag (Caine, Stonelake, 2002; Athale, Moghrabi, 2010). Third, that cancer cells are able to

manufacture and release procoagulant substances or to interact with endothelial cells, leading to activation of coagulation (Caine, Stonelake, 2002). Thus, indicating the increase in the concentrations of VWF:Ag caused by to the outcomes of the disease itself and could be a reflection of endothelial stimulation (Albayrak, Gürsel, 2013). Our results agreed with both ElSherif and Athale study. They reported that the level of VWF:Ag was significantly correlated with blast count (Athale, Moghrabi, 2010; El Sherif, Narouz, 2014).

When plasma levels of VWF:Ag were determined following induction therapy (D35), we noticed that children who had PB showed significant elevation in levels of VWF:Ag whereas patients without peripheral blasts had no significant variation in VWF:Ag levels (64.18 vs 49.30;  $P=0.652$ ). Increase level of VWF:Ag can be elucidated by chemotherapy agents which cause adverse impacts on endothelium, and induce endothelial malfunction which causes an increase VWF:Ag. (Jarvela, Niinikoski, 2013). Also, the induction chemotherapy (day 35) leads to reactivation of coagulation via extrinsic pathway probably due to the procoagulant substances released by lysed blasts and related with raised coagulation events observed in ALL patients (Albayrak, Gürsel, 2013). Many studies have advocated that steroid therapy used in ALL protocol are implicated in clotting activation by increasing the synthesis and secretion of VWF (Hatzipantelis, Athanassiou-Metaxa, 2010). Prednisolone causes a raise in VWF:Ag levels whereas dexamethasone has a moderate effect (Brotman, Girod, 2006). In addition, doxorubicin used in the first phase of therapy triggers cell suicide process of endothelium (Dengel, Ness, 2008), and the combination of prednisolone and asparaginase led to elevation in VWF:Ag (Payne and Vora 2007). Our results approved both study of Emmanouel, (Hatzipantelis, Athanassiou-Metaxa, 2010) and study of Schneider (2010), who showed that VWF:Ag concentrations increased during induction therapy with PRD, VCR, DNM, and ASP (Schneider, Dreden, 2010). Our results disagreed with both ElSherif study and Athale study. They have noted decrease level of VWF:Ag in children with blast cells at day 8 and day 3 of steroid therapy which used alone. (El Sherif, Narouz, 2014). In our study, induction therapy (day 35) consists of Steroid therapy in combination with other agents and treatment protocol was different that children enrolled on BFM90 protocol which used PRED 60 mg/m<sup>2</sup> instead of dexamethasone (DMZ) which given at a dose of 6 mg/m<sup>2</sup>/d in ElSherif study or methylprednisolone monotherapy (MpMT) (32 mg/m<sup>2</sup>/d) in Athale study. So we concluded that dose or duration steroid therapy, various kinds of steroids and of their combination with other drugs all of these factors effect on VWF:Ag concentrations.

#### 4. CONCLUSION

The significantly elevated levels of VWF in ALL patients through the critical stage and remission of the disease approve the occurrence of endothelial malfunction in ALL.

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