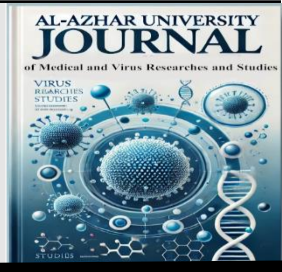
Al-Azhar University Journal
for Medical and Virus Research
and Studies




# Vanin– 1 Protein Level Among Primary Immune Thrombocytopenic Purpura Cases

**Mohamed Ramadan Izzaldin[1], Walaa Ali El-Salakawy[2] and Rasha Magdy Mohamed said [2]**

**[1]Department of Clinical Pathology, Faculty of Medicine, Al-Azhar University, Assiut, Egypt.**

**[2]Department of Internal Medicine and Clinical Hematology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.**

***E-mail: m1100200275@gmail.com**

## Abstract

The development of chronic ITP may be linked to vanin-1 overexpression. When blood mononuclear cells were subjected to oxidative stress inducers, VNN1 was markedly upregulated and PPARg was markedly downregulated. These results show that VNN1 serves as a peripheral blood oxidative stress sensor. The current study aimed to measure Vanin -1 protein level by ELISA as a predictive factor in patients with primary immune thrombocytopenic purpura. A Comparative, case-control study, included 30 primary ITP cases along with 20 healthy subjects as a control group. A comprehensive clinical examination, a complete history taking, and laboratory investigation (Vanin-1 protein level by ELISA) were performed on all patients. The level of Vanin-1 level was significantly higher among ITP cases compared to the controls. There were no statistically significant correlations between Vanin-1 levels and laboratory findings among ITP patients except for HCT (%) which shows a significant negative moderate correlation with Vanin-1. Analysis of the ROC curve shows that Vanin-1 can discriminate ITP patients from healthy controls, with a highly significant difference. Using coordinate points of the above ROC curve, the most relevant level of Vanin-1 is 0.7 to differentiate ITP patients from healthy controls, with a sensitivity of 100%, and a specificity of 95%. Vanin -1 can be used as a predictive factor to differentiate ITP cases from healthy non-diseased subjects.

**Keywords:** Cystic lesions – MDCT – Chest.

## 1. Introduction

Vanin-1, also known as vascular non-inflammatory molecule-1, is an ectoenzyme that exhibits pantetheinase activity. It is strongly expressed in several organs, including the kidney, gut, and liver, both at the gene and protein levels. Its pantetheinase activity plays a crucial role in the following ways: Pantetheine is broken down by vanin-1 into cysteamine and pantothenic acid, which is a building block

for coenzyme A [2]. By opposing peroxisome proliferator-activated receptor-g (PPARg), vanin-1, an epithelial glycosylphosphatidylinositol inositol-anchored pantetheinase, is known to play a role in the oxidative stress response and permit the production of inflammatory mediators in murine intestinal epithelial cells [1-4].

It has been demonstrated that patients with juvenile chronic ITP during the acute phase and patients with treatment-resistant chronic ITP have elevated expression of the VNN1 gene, which is known to be an anti-inflammatory checkpoint in numerous inflammatory contexts and cell types [5].

Overexpression of vanin-1 may be associated with progression to chronic ITP. VNN1 was dramatically upregulated and PPARg was dramatically downregulated when blood mononuclear cells were exposed to oxidative stress inducers, suggesting that VNN1 functions as a peripheral blood oxidative stress sensor [5]. In another study, it was shown that adult ITP patients' blood Vanin-1 protein levels displayed a highly distinct and unusual pattern when compared to healthy controls. Furthermore, Vanin-1 levels have the high sensitivity and specificity to distinguish between ITP patients who react and those who do not [6].

An especially noteworthy finding was that individuals with freshly diagnosed ITP had higher amounts of vanin-1 protein than those with chronic ITP. Glycosylphosphatidyl inositol vanin-1 is attached to cell membranes and is not present in serum [7]. Further studies are needed to clarify the role of Vanin -1 in the pathogenesis and chronicity of primary ITP. The current study aimed to measure Vanin -1 protein level by ELISA as a predictive factor in patients with primary immune thrombocytopenic purpura.

## 2. Patients and Methods

**Study design:** A comparative, case-control study.

**Study setting:** Ain Shams University Hospitals, Clinical Hematology of Internal Medicine Department.

**Patients:** A total of 30 primary ITP cases were included in the study, along with 20 healthy subjects as a control group.

### 2.1 Inclusion criteria

- Newly diagnosed ITP patients
- Age > 18 years.

### 2.2 Exclusion Criteria

- Patients with chronic ITP
- Patients with thrombocytopenia due to (viral and bacterial infections, collagen diseases, myelodysplastic disorders, aplastic anemia, etc…).

### 2.3 Study tools

All patients were subjected to the following at the time of recruitment:

1. Full history taking (current acute or chronic inflammation, history of liver disease in detail, kidney disease, medications, alcohol consumption, previous viral infection (hepatitis C virus (HCV), human immune deficiency virus (HIV|), malignancy, myeloproliferative disorder, lymphoproliferative disorder, plastic anemia, MDS and autoimmune disorder….)
2. Thorough clinical examination (hepatosplenomegaly, lymphadenopathy, anemia, vital, …..)
3. Laboratory investigations that included:
   A. Complete blood count (CBC).
   B. Bone marrow aspirates in selected patients.
   C. Liver functions test.
   D. Kidney functions test.
   E. CRP, ESR.
   F. Viral markers.
   G. ANA Assay.
   H. Vanin -1 protein level by ELISA.

## 2.4 Ethical considerations

Every research subject provided signed informed permission. The study complies with the requirements of the Helsinki Declaration and was approved by the Ain Shams University Ethical Committee Board.

## 2.5 Specimen collection

Using sterile plastic syringes, 6.0 ml of venous blood was taken from each individual via venipuncture and divided into two equal portions:

- To estimate the whole blood image, 2.0 ml was put to the ethylene diamine tetraacetic acid (EDTA) tube and well mixed by gently turning the tube upside down many times.
- For the purpose of estimating ESR, add 2.0 ml of sodium citrate to a tube and mix well by gently turning the tube upside down several times.

- The serum plain tube was filled with 2.0 ml, left to clot at room temperature, then centrifuged for 15 minutes at 4000 rpm. Serum Vanin 1 Assay, Liver Function Test, Kidney Function Test, Viral Hepatitis Markers, Serum ANA, CRP, and ESR are all performed on the separated serum.

## 2.6 Assay of vanin – 1

The assay of Vanin-1 was done as discussed in the manufacturer instructions as Sandwich enzyme-linked immunoassay.

## 2.7 Reagent preparation

- Before using, let all samples and reagents come to room temperature (~ 25℃) and thoroughly mix by gently inverting.
- Standards: Immediately before the test, reconstituted the lyophilized standard with 1.0 ml Standard/Sample Diluent (R1) and allowed it to dissolve for at least 15 minutes, gently stirring it every now and then.
- Then, prepare a set of standards using the stock and the standard/Sample Dilution (R1) as shown in Table .1.

The prepared standards are ready to use and should be used within 1 hour after preparation.

- Working Biotin Conjugate Antibody: Before using, dilute the concentrated biotin conjugate antibody (100 X) 1:100 times in the biotin conjugate antibody diluent (R2). Then, add 20 ul of the concentrated biotin conjugate antibody and 1980 ul of the Biotin Conjugate Antibody diluent (R2) to create 2000 ul of working biotin conjugate antibody.
- Working Streptavidin – HRP: To create working streptavidin-HRP right before use, dilute 1:100 of the concentrated streptavidin-HRP (100 X) in Streptavidin-HRP diluent (R3) (Add 20 ul of concentrated streptavidin-HRP + 1980 ul Streptavidin-HRP diluent (R3) to prepare 2000 ul working streptavidin-HRP).
- Wash Buffer: Preparing the working wash buffer right before usage requires diluting wash buffer concentrate (1:25; add 16 ml of concentrate to 384 ml of deionized water or D.W. to make 400 ml of wash buffer).

**Table (1):** Prepare samples.

| Tube No. | Volume of Standard | Standard/Sample Diluent | Concentration |
|---|---|---|---|
| 1 | Stock | - | 13.5 ng/ml |
| 2 | 250 µl of tube 1 ( 9 ng/ml ) | 250 µl | 9.0 ng/ml |
| 3 | 250 µl of tube 2 ( 6 ng/ml ) | 250 µl | 6.0 ng/ml |
| 4 | 250 µl of tube 3 ( 3 ng/ml ) | 250 µl | 3.0 ng/ml |
| 5 | 250 µl of tube 4 ( 1.5 ng/ml ) | 250 µl | 1.5 ng/ml |
| 6 | 250 µl of tube 5 ( 0.75 ng/ml ) | 250 µl | 0.75 ng/ml |
| 7 | 250 µl of tube 6 ( 0.0 ng/ml ) | 250 µl | 0.0 ng/ml |

## 2.8 Procedural steps

1. Allow all specimens and reagents to reach **R.T. (~ 25ºC)** before use.
2. Mark the micro titration strips to be used.
3. As instructed before, prepare all reagents, working standards, and samples.
4. Add **350 ul** wash buffer to each well for **40 seconds**, then aspirate the solution. Repeat this step for another two times for a total of **three washes**.
5. Add **100 ul** of standard/sample Diluent (R1) in the first well as a blank.
6. Add **100 µl** of each standard concentration, into the corresponding wells.
7. Add **100 µl** of each sample into the corresponding wells.
8. Cover with the adhesive sealer. Incubate the plate at **37 C** for **2 hours**.
9. Start preparing the Biotin Conjugate Antibody (100 X) working solution just **15 minutes** before the end of incubation time.
10. Aspirate the solutions by the end of the incubation time.
11. Wash the wells **3 times (As in step 4)**. After the final wash, invert and tap the plate strongly against a paper towel.
12. Add **100 µl** of working conjugate **(that was prepared in step 9)** into each well.
13. Cover with new adhesive sealer. Incubate the plate at **37 C** for **1 hour**.
14. Start preparing the Streptavidin-HRP concentrated (100 X) working solution just **15 minutes** before the end of incubation time.
15. Aspirate the solutions by the end of the incubation time.
16. Wash the wells **3 times (As in step 4)**. After the final wash, invert and tap the plate strongly against a paper towel.
17. Add **100 µl** of the Streptavidin-HRP working solution **(that was prepared in step 14)** into each well.
18. Cover with new adhesive sealer. Incubate the plate at **37 C** for **30 minutes**.
19. Aspirate the solutions by the end of the incubation time.
20. Wash the wells **3 times (As in step 4)**. After the last wash, flip the plate over and firmly tap it on a paper towel.
21. Add **90 ul** of TMB Substrate into each well
22. Incubate the plate at **37 C** for **30 minutes (Away from light)**
23. During the incubation time, **turn on** the reader to warm up
24. Add **50 µl** of the Stop solution**.**
25. Determine the optical density (OD) of each well at **450 nm** on a microplate reader within **5 minutes** after adding the stop solution.

## 2.9 Data management and statistical analysis

- SPSS, version 25 was used to tabulate and statistically analyze the data (SPSS Inc., Chicago, IL).
- The mean, standard deviation, median, and interquartile range were used to characterize quantitative data.
- The qualitative information was presented as percentages (%) and frequencies (n).
- The Independent t-test was used for comparing quantitative data between the 2 groups.
- The Chi-square test was used to test the relation between 2 qualitative variables.
- To establish a link between nonparametric quantitative variables, the Pearson correlation coefficient was employed.
- Roc Curve analysis was used to detect the area under the curve and the cut-off point for Vanin-1 for discrimination between groups.
- The Kappa agreement test was used to test the agreement between the results of Vanin-1 results after using the detected cut-off and the diagnosis of groups.

- P-value ≤ 0.05 was considered significant.

## 3. Results

The current study included 30 ITP cases and 20 healthy controls. The mean age of the study cases was 29 years, compared to 31 years among controls, with no significant difference. ITP cases showed female predominance (63.3%) compared to controls (50%).
The manifestations of ITP cases, the most common was anemia with bleeding (36.7%), bleeding alone (30%), anemia alone (16.7%). Other cases were accidentally discovered with no symptoms.
About red blood cell (RBCs) parameters, we found that there are statistically significant differences between hemoglobin level (HB) and hematocrit value (HCT) but non-significant differences between MCV and RBCs count. There were statistically significant differences in platelet parameters distribution between ITP patients and controls. Regarding the white blood cell parameters distribution between ITP and controls, we found that there is a statistically significant difference regarding monocyte series, but not regarding WBCs count (Table .1).
The difference between the cases and controls regarding the mean Vanin-1 level, we found that the level of Vanin-1 level was significantly higher among ITP cases compared to the controls Table .2.
We found that there were no statistically significant correlations between Vanin-1 levels and laboratory findings among ITP patients except for HCT (%) which shows a significant negative moderate correlation with Vanin-1. Among controls, we found that the only significant correlation was with RBCs count which shows a significant negative moderate correlation with Vanin-1 Table .3.
Analysis of the ROC curve shows that Vanin-1 can discriminate ITP patients from healthy controls, with a highly significant difference (p value <0.001) and a high area under the curve of around 0.997. Using coordinate points of the above ROC curve, the most relevant level of Vanin-1 is 0.7 to differentiate ITP patients from healthy controls, with a sensitivity of 100%, and a specificity of 95% Figure .1.
Table .4 shows the validity of Vanin-1 results for discrimination between ITP and healthy controls by using the cut-off value (0.7) where the values lower than (0.7) represent healthy controls while the values equal to more than (0.7) represent ITP.

**Table (2):** CBC parameters distribution between ITP and controls.

| RBCs | | ITP | Control | P value |
|---|---|---|---|---|
| HB (g/dl) | Mean ± SD (min-max) | 11.1±1.7 (7.8-14) | 13.7±1.5(11.5-16.5) | <0.001* |
| | Median (IQR) | 11.5 (9.6-12.5) | 13.5 (12.3-14.8) | |
| RBCs MCV | Mean ± SD (min-max) | 84±9 (69-111) | 81±4 (70-89) | 0.214 |
| | Median (IQR) | 83 (76-89) | 81 (80-84) | |
| RBCs | Mean ± SD (min-max) | 4.8±3.9 (2.8-25) | 4.9±0.5 (3.8-5.6) | 0.908 |
| | Median (IQR) | 4.1 (3.7-4.7) | 5 (4.5-5.4) | |
| HCT (%) | Mean ± SD (min-max) | 33±7.6 (3.9-44) | 39.7±4 (36-48) | 0.001* |
| | Median (IQR) | 35.3 (31-37) | 38 (36.6-42.5) | |
| Platelets | | | | |
| Platelet count | Mean ± SD (min-max) | 54.2±37.8(1-130) | 232.1±57.3(164-375) | <0.001* |
| | Median (IQR) | 43 (21-82) | 222 (185-259.5) | |
| Plat_LCR (%) | Mean ± SD (min-max) | 37.5±13.6(21-63) | 27.8±8.9 (17-48) | 0.004* |
| | Median (IQR) | 36.6 (25-44) | 26.5 (20.5-31) | |
| WBCs | Mean ± SD (min-max) | 7.2±2.3 (2.5-13) | 7.2±1.7 (4-10) | 0.995 |
| | Median (IQR) | 6.9 (5.6-8.5) | 7.3 (5.9-8.5) | |
| Monocytes series (%) | Mean ± SD (min-max) | 7.6±3.6 (2-15) | 5.4±2.5 (1-10) | 0.013* |
| | Median (IQR) | 7 (5-9.9) | 5.6 (3.5-7) | |

**Table (3):** Comparison between ITP and controls regarding Vanin-1 level.

| | | ITP | Control | P value |
|---|---|---|---|---|
| **Vanin-1 level** | **Mean±SD (min-max)** | 3.8±1.8(1.2-9) | 0.2±0.3(0-1.5) | **<0.001*** |
| | **Median (IQR)** | 3.7 (2.7-4.6) | 0.1 (0.1-0.2) | |

**Table (4):** Correlation between Vanin-1 and laboratory findings among patients with ITP and controls.

| Item | Among ITP cases | | Among controls | |
|---|---|---|---|---|
| | r | P value | r | P value |
| **Platelets count** | -0.176 | 0.352 | -0.242 | 0.304 |
| **Platelet LCR%** | 0.014 | 0.940 | -0.054 | 0.821 |
| **WBCs** | -0.176 | 0.354 | -0.125 | 0.599 |
| **HG (g/dl)** | 0.001 | 0.996 | -0.380 | 0.099 |
| **RBCs MCV** | 0.049 | 0.797 | 0.213 | 0.367 |
| **Monocyte series (%)** | -0.330 | 0.075 | -0.251 | 0.286 |
| **RBCs** | -0.292 | 0.117 | -0.506 | **0.023*** |
| **Hematocrit (%)** | -0.419 | **0.021*** | -0.233 | 0.324 |
| **CRP** | 0.056 | 0.767 | 0.373 | 0.106 |
| **ALT** | -0.333 | 0.072 | -0.015 | 0.951 |
| **Creatinine** | -0.142 | 0.455 | -0.047 | 0.843 |
| **ESR** | -0.152 | 0.422 | -0.083 | 0.730 |

**r: Pearson Correlation**

**Table (5):** Validity of Vanin-1 for discrimination between ITP and healthy controls.

| Sensitivity | Specificity | Positive predictive value | Negative predictive value |
|---|---|---|---|
| 100% | 95% | 96.8% | 100% |

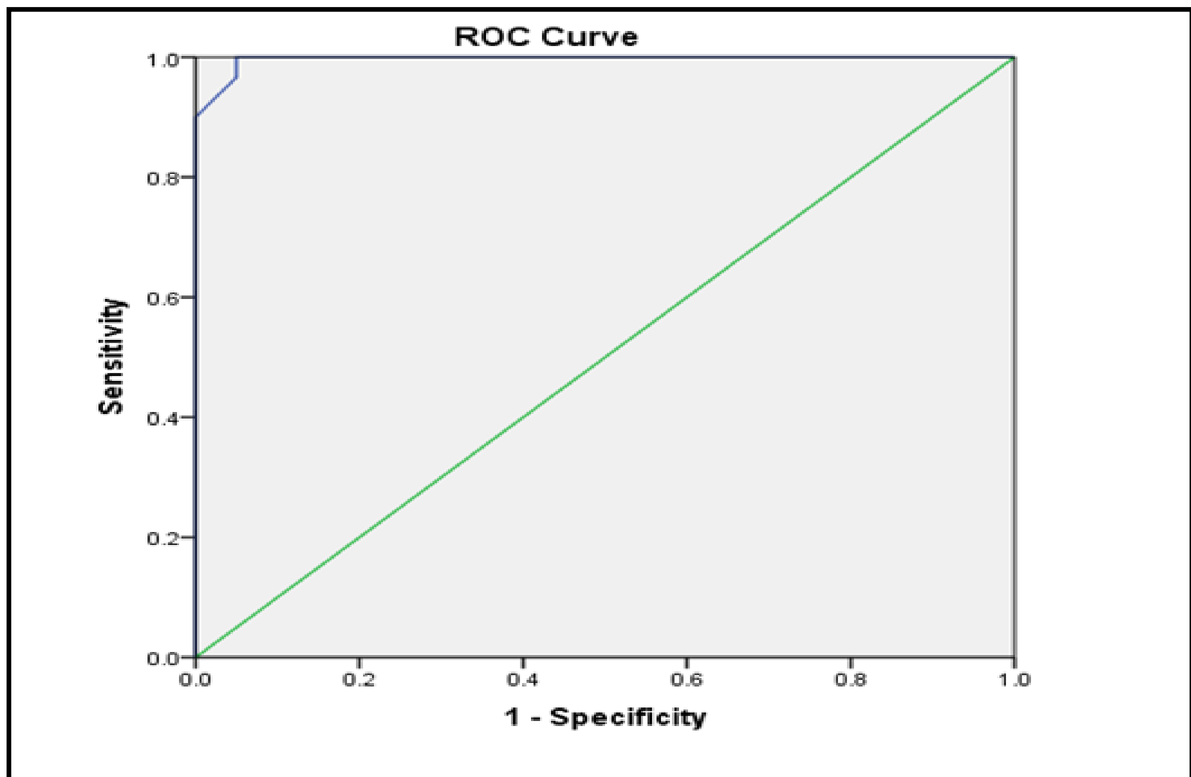



**Figure (1):** ROC curve for Vanin-1 for discrimination between ITP and healthy controls.

## 4. Discussion

The development of chronic ITP may be linked to Vanin-1 overexpression. VNN1 was dramatically upregulated and PPARg was dramatically downregulated when blood mononuclear cells were exposed to oxidative stress inducers, suggesting that VNN1 functions as a peripheral blood oxidative stress sensor [5].

The symptoms of ITP, a hematological autoimmune illness, include bleeding and a platelet count below $100\times10^9$/L. The exact mechanisms by which the host immune response turns against its own system (autoimmunity) and results in ITP are currently unclear, while there are many different potential causes of ITP. More and more evidence suggest that the main process behind immunological tolerance pathogenesis (ITP) is the unbalanced interaction between effectors and regulatory immune cells [8-10].

The current study aimed to measure Vanin -1 protein level by ELISA in patients with newly diagnosed primary immune thrombocytopenic purpura.

The mean age of the study population was around 30 years. In the study of **Elsalakawy et al.,** [6], the median age of the study population was around 31-35 years among ITP patients and 36 years among healthy controls. The study of **Eissa et al.,** [11] was done on children with ITP, with a mean age of 7-8 years.

In the current study there was female predominance among the ITPs group, while the control group were equally divided. The study of **Elsalakawy et al.,** [6] showed a very high female predominance as over 90% of the ITP cases and healthy controls were females. On the other hand, the study of **Eissa et al.,** [11] showed that the majority of their cases were males (60% among controls and over 55% among ITP cases).

We found that there are statistically significant variations in HB and HCT among RBC characteristics. Between ITP patients and controls, there were statistically significant variations in the distribution of PLT parameters. We found a statistically significant variation in the distribution of WBCs parameters between ITP and controls with respect to the monocyte series. In the study of **Eissa et al.,** [11], the mean hemoglobin level was 13.4 gm/dL among control subjects compared to 10.88 gm/dL among ITP patients. The study of **Elsalakawy et al.,[6]** showed that the median platelet count among ITP patients was 61 x $10^3$/ $mm^3$ and 187 x $10^3$/ $mm^3$ among healthy controls. In the study done by **Eissa et al.,** [11], the mean platelet count was significantly lower among ITP patients (42.7 x $10^3$/ $mm^3$) compared to controls (254.9 x $10^3$/ $mm^3$). The study of **Eissa et al., [11]** showed that the mean WBCs were significantly higher among ITP Patients 8.17 x $10^3$/ $mm^3$ compared to 5.84 x $10^3$/ $mm^3$ among controls.

the Vanin-1 levels distribution across all groups, we found that it showed higher levels in ITP patients compared to healthy controls. In the study of **Elsalakawy et al., [6]** the Vanin-1 level showed an inverse relation to the platelet count, being higher with lower platelet count, and significantly lower levels of Vanin-1 among healthy controls compared to ITP patients, and among ITP responders compared to ITP non-responders. In the study of **Eissa et al., [11]**, the mean level of Vanin-1 was significantly higher among ITP cases (4.92 ng/ml) compared to 1.13 ng/dl among controls. Also, they found that the mean Vanin-1 level was significantly related to the response and onset of ITP, being highest among newly diagnosed ITP (9.08 mg/dl); compared to non-responder chronic ITP (1.16 ng/dl) and lowest among responder chronic ITP (0.43 ng/dl). Regarding the relations between Vanin-1 levels and clinical and laboratory data of the study population, they found that there were non-significant relations among responder and non-responder chronic ITP

patients; while among newly diagnosed ITP; they found that Vanin-1 was inversely related to both age and platelet levels.

the exception of HCT, which exhibits a substantial negative moderate connection with Vanin-1, there were no statistically significant negative weak correlations found between Vanin-1 levels and laboratory results. among fit reference points. For health controls, there was no statistically significant link found between Vanin-1 levels and laboratory results; nevertheless, there is a substantial negative moderate correlation found between Vanin-1 and RBCs count. We found that Vanin-1 can distinguish ITP patients from healthy controls with a highly significant difference, according to ROC curve analysis.

In the study of **Zhang et al.,** [5], chronic non-responders ITP showed a substantial rise in serum VNN1 levels in comparison to chronic responders. Also, they found that the level of VNN1 was significantly higher among chronic ITP compared with self-limited acute ITP, suggesting a role for oxidative stress in the pathogenesis of chronic ITP.

In the study of **Elsalakawy et al.,** [6], ROC curve analysis showed that Vanin-1 could be used to distinguish ITP patients from healthy controls, with a sensitivity of 90.6% and a specificity of 93.7%. Also, Vanin-1 can differentiate newly diagnosed ITP from healthy controls, with a sensitivity of 100% and a specificity of 93.7%. Furthermore, they found that Vanin-1 could differentiate ITP non-responders from ITP responders with a sensitivity and specificity of 100%.

In the study done by **Eissa et al.,** [11], ROC curve analysis showed that Vanin-1 could be used as a predictive factor to differentiate ITP patients from controls, at a cut-off value of 0.6 ng/dl, with a sensitivity, specificity, positive and negative predictive values of 100%.

## 5. Conclusion

Vanin -1 can be used as a predictive factor to differentiate ITP cases from healthy non-diseased subjects.

**Funding Sources:** There was no support for this study from any governmental, private, or non-profit organization.

**Conflicts of interest:** No competing interest

## References

1. Berruyer C, Pouyet L, Millet V, Martin FM, LeGoffic A, Canonici A, et al. Vanin-1 licenses inflammatory mediator production by gut epithelial cells and controls colitis by antagonizing peroxisome proliferator-activated receptor gamma activity. The Journal of experimental medicine. 2006;203(13):2817-27.

2. Aurrand-Lions M, Galland F, Bazin H, Zakharyev VM, Imhof BA, Naquet P. Vanin-1, a novel GPI-linked perivascular molecule involved in thymus homing. Immunity. 1996;5(5):391-405.

3. Pitari G, Malergue F, Martin F, Philippe JM, Massucci MT, Chabret C, et al. Pantetheinase activity of membrane-bound Vanin-1: lack of free cysteamine in tissues of Vanin-1 deficient mice. FEBS Lett. 2000;483(2-3):149-54.

4. Berruyer C, Martin FM, Castellano R, Macone A, Malergue F, Garrido-Urbani S, et al. Vanin-1-/- mice exhibit a glutathione-mediated tissue resistance to oxidative stress. Molecular and cellular biology. 2004;24(16):7214-24.

5. Zhang B, Lo C, Shen L, Sood R, Jones C, Cusmano-Ozog K, et al. The role of vanin-1 and oxidative stress-related pathways in distinguishing acute and

chronic pediatric ITP. Blood. 2011;117(17):4569-79.

6. Elsalakawy WA, Ali MA, Hegazy MG, Farweez BA. Value of vanin-1 assessment in adult patients with primary immune thrombocytopenia. Platelets. 2014;25(2):86-92.

7. Huang H, Dong X, Kang MX, Xu B, Chen Y, Zhang B, et al. Novel blood biomarkers of pancreatic cancer-associated diabetes mellitus identified by peripheral blood-based gene expression profiles. The American journal of gastroenterology. 2010;105(7):1661-9.

8. Sahi PK, Chandra J. Immune Thrombocytopenia: American Society of Hematology Guidelines, 2019. Indian pediatrics. 2020;57(9):854-6.

9. Neunert C, Terrell DR, Arnold DM, Buchanan G, Cines DB, Cooper N, et al. American Society of Hematology 2019 guidelines for immune thrombocytopenia. Blood Adv. 2019;3(23):3829-66.

10. McKenzie CG, Guo L, Freedman J, Semple JW. Cellular immune dysfunction in immune thrombocytopenia (ITP). British journal of haematology. 2013;163(1):10-23.

11. Eissa M, Zidan A, Omran AA. The Predictive Value of Vanin-1 in Pediatric Immune Thrombocytopenia. The Egyptian Journal of Hospital Medicine. 2021;85(2):3737-41.