

**PERLS' PRUSSIAN BLUE POSITIVITY IN EXFOLIATED BUCCAL CELLS OF  $\beta$  THALESSEMIA MAJOR PATIENTS AND ITS CORRELATION WITH SERUM FERRITIN**Sonam Gupta<sup>1</sup>, V. K. Trichal<sup>2</sup>, Reeni Malik<sup>3</sup>, R. K. Nigam<sup>4</sup>, Rajni Choudhary<sup>5</sup>, Atul Shrivastava<sup>6</sup>**HOW TO CITE THIS ARTICLE:**

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**ABSTRACT: BACKGROUND:** Iron absorption is carefully regulated to maintain equilibrium between the absorption and the body loss of iron. Thalassemia is a genetic disorder of hemoglobin synthesis requiring regular blood transfusion leading to iron overload in the body which leads to morbidity and mortality in these patients. **AIMS AND OBJECTIVES:** To demonstrate iron overload at an early stage by exfoliative cytology using Perls' Prussian blue method and to compare with that of serum ferritin levels of those patients. **MATERIALS AND METHODS:** Smears were obtained from buccal mucosa of 60  $\beta$  thalassemia major patients who were undergoing repeated blood transfusion and 30 healthy subjects as control. Scrapings were obtained from the buccal mucosa and stained with Perls' Prussian blue stain and were examined under light microscope. **RESULT:** Perls' positivity was observed in 61.6% of  $\beta$  thalassemic patients with a moderately positive correlation to serum ferritin levels. **CONCLUSION:** Exfoliative cytology is a simple painless and a non-invasive technique therefore it can be used as a diagnostic tool in demonstration of iron overload in thalassemic patients.

**KEYWORDS:** Iron overload, thalassemia, exfoliative cytology, Perls' Prussian blue reaction.

**INTRODUCTION:** Minerals are inorganic elements that are essential for life. Among them iron is an essential nutrient which are required by human cell. Approximately 4.5 g of iron can be found in an average adult man most of which is contained in the hemoglobin molecule and in other haem containing proteins.<sup>1</sup> Under physiologic conditions the concentration of iron in the human body is carefully distributed between functional, transport and storage components.<sup>2</sup>

In 1925 Thomas Cooley and Pearl Lee described a form of severe anemia associated with splenomegaly and characteristic bony changes. As all earlier cases were reported in children of the Mediterranean origin, the disease was later named thalassemia, from the Greek word for sea Thalessa.<sup>3</sup>

In Thalassemia major the production of beta globin chains is severely impaired because both beta globin genes are mutated which results in a severe or total suppression of beta chain synthesis. Beta thalassemia major is a life threatening condition characterized by severe anemia, hepatosplenomegaly, growth retardation endocrine dysfunction and skeletal changes due to hypertrophy and expansion of the hematopoietic marrow.<sup>4</sup>

In beta thalassemia there is ineffective erythropoiesis because of excess of alpha globin chains & increased breakdown of red cells which leads to severe anaemia.<sup>5</sup> Therefore they require regular blood transfusions. The goal of transfusion includes correction of anemia, suppression of erythropoiesis and inhibition of gastrointestinal absorption of iron.

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In patients who are not receiving transfusions, abnormally regulated iron absorption results in increasing in body iron burden ranging from 2-5 g/yr. Regular transfusion may double this rate of iron accumulation.

After approximately 1 yr. iron begins to be deposited in parenchymal tissue where it may cause substantial toxicity as compared with that within reticuloendothelial cells.

Storage iron occurs in two forms- ferritin and hemosiderin. In states of iron overload hemosiderin increases to a greater degree than ferritin and becomes the dominant form.<sup>6</sup> Ferritin although may be present in the cells cannot be visualized under light microscope. Hemosiderin because of its large size can be visualized under light microscope as blue coloured granules in the cytoplasm of the cells.<sup>7</sup>

In our study exfoliated cells from the buccal mucosa of 60  $\beta$  thalassemia major patients comprising the study undergoing transfusions and a control group of 30 normal individuals who had no confirmed acute and chronic liver damage, malignancy and megaloblastic anemia were considered and the smears obtained were stained with Perls' Prussian blue stain.

### AIMS AND OBJECTIVES:

1. To demonstrate iron overload in an early stage in the exfoliated buccal cells of beta thalassemia major patients.
2. To correlate Perls' Prussian blue staining with serum ferritin levels.

**MATERIALS AND METHODS:** The study was conducted after approval from institutional ethical committee. The study group comprised of 60 hemoglobin electrophoresis confirmed beta thalassemia major patients ranging in the age group of 1- 16 yrs. receiving regular blood transfusion. The control group comprised of 30 both clinically & hematologically healthy individuals in the age range of 1-16 yrs. who had no confirmed acute and chronic liver damage, malignancy and megaloblastic anemia.

For the preparation of the smears clean, fresh and dry glass slides were used. Scrapings were obtained from the subject's mouth by using a wet wooden spatula, by using a gentle scraping motion with little pressure over buccal mucosa of both the study and control groups. The scraps were smeared into the centre of the glass slides & immediately fixed in 90% alcohol for one hour, then stained with Perls' Prussian blue stain (which consist of Potassium ferrocyanide and HCl) which reacts with the ferritin in the cells to form blue coloured granules.<sup>8</sup>

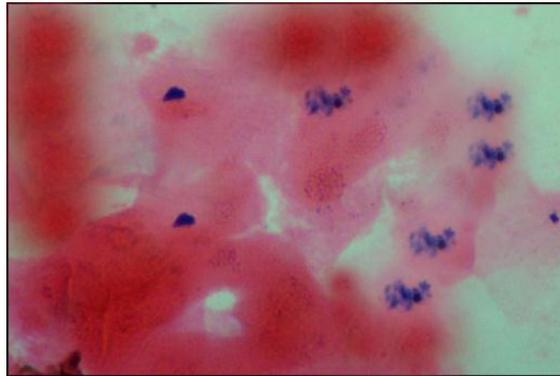
Blue granules indicated the presence of iron overload. The stained smears were examined under 4x, 10x and 100x magnifications to study the presence or absence of blue coloured granules in the cells.

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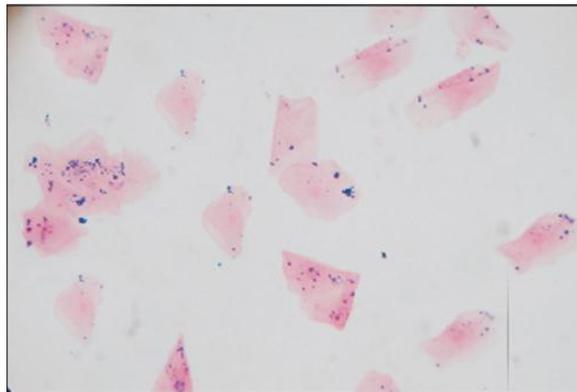
### RESULT:

Figure 1: Smear at higher magnification showing clumps of blue colored granules (Perls' Prussian blue stain, x 400).



**Figure 1**

Figure 2: Smear at higher magnification showing dispersed blue colored granules (Perls' Prussian blue stain, x400)



**Figure 2**

Figure 3: Smear at higher magnification showing clumps of blue colored granules (Perls' Prussian blue stain, x 400)



**Figure 3**

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GROUP	PERLS PRUSSIAN BLUE REACTION				t- Test	P Value
	NEG ATIVE		POSITIVE			
	NUMBER	%	NUMBER	%		
PATIENTS	23	38.2%	37	61.6%	27.3	<0.0001
CONTROL	29	96.6%	1	3.3%		

**TABLE 1 : Comparison of Perls' Prussian blue Reaction in  $\beta$ -thalassemic patients and control subjects**

	PATIENTS				CONTROL				t- Test	P Value
	MEAN	SD	MIN	MAX	MEAN	SD	MIN	MAX		
SERUM FERRITIN	586.3	184.6	250	790	56	28	18	90	15.51	<0.0001

**TABLE 2: Comparison of serum ferritin levels in  $\beta$ -thalassemic patients and control subjects**

Serum ferritin	Perls' Prussian blue staining positivity
Correlation coefficient	0.76
P Value	<0.0001 Sig
N	60

**TABLE 3: Correlation of Perls' Prussian staining positivity and serum ferritin levels in  $\beta$  - Thalassemic patients**

Serum ferritin	Number of Blood Transfusions
Correlation coefficient	0.68
P value	<0.0001 sig
N	60

**TABLE 4: Correlation between number of blood transfusions and serum ferritin levels in  $\beta$  - Thalassemic patients**

**DISCUSSION:** Perls' Prussian blue reaction is considered to be the first classical histochemical reaction carried out, and is widely applied in the field of haematology.<sup>9</sup> We have applied this technique to exfoliated buccal cells.

In our study, exfoliated cells from the buccal mucosa of 37 of the 60 thalassemic patients (61.6%) group revealed positivity for Perls' Prussian blue reaction. Chi -square test was applied and the P value was <0.0001 and the test was considered significant. And one of the control subjects showed false Perls' Prussian blue positivity.

Our results were similar to those observed by Nandprasad et al (2010) who recorded 65 % Perls' positivity although these were lower than Atul A Bhat et al (2013) who recorded 71.7% Perls' positivity, Harika Chittamsetty et al (2013) who observed 72.5% and Gururaj and Sundaram et al (2004) who observed 100% Perls' positivity.

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SL. No.	Study	year	Positive cases	% positivity
1)	Gururaj and Sundaram et al	2004	10/10	100%
2)	Nandprasad et al <sup>2</sup>	2010	65/100	65%
3)	Atul A Bhat et al <sup>10</sup>	2013	43/60	71.7%
4)	Harika Chittamsetty et al <sup>11</sup>	2013	29/40	72.5%
5)	Present study	2014	37/60	61.6%

**Tables 5: Comparative study of similar studies**

The serum ferritin was higher in our study groups as compared to control group.

Our results showed a moderately positive correlation between Perls' Prussian blue positivity and serum ferritin which indicates that the positive staining in exfoliated buccal cells may give a clue of increased serum ferritin levels and thus indicating iron overload in the body tissues.

**CONCLUSION:** Measurement of iron concentration in a liver biopsy specimen is a reference method for assessing the body iron stores. The discomfort and risk associated with the liver biopsy procedure as well as cost factor associated with MRI as compelled us to search for a simple and a cheaper technique.

Oral exfoliative cytology is a simple and non-invasive technique which can be used for screening and diagnostic tool for assessing iron status in thalassemia patients undergoing repeated blood transfusions.

Further advancement in this field is advocated to establish this technique as a screening tool & also to assess complications associated with iron overload.

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