

## Comparison of nitric oxide levels in patients undergoing surgery in absence and deliverance of blood transfusion in association with hemoglobin

Ragini Singh<sup>1</sup>, Veena Singh Ghalaut<sup>2</sup>, Seema Lekhwani<sup>3\*</sup>, Isha Malik<sup>4</sup>, Vaibhav Vishal<sup>5</sup>

<sup>1,3</sup>Assistant Professor, <sup>2</sup>Senior Professor and HOD, <sup>4</sup>Associate Professor, <sup>5</sup>Post-graduate Student, <sup>1</sup>Dept. of Blood Transfusion, <sup>2-5</sup>Dept. of Biochemistry, <sup>1</sup>Bhagat Phool Singh, Govt. Medical College for Women, Khanpur Kalan, Sonapat, Haryana, <sup>2-5</sup>Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India

**\*Corresponding Author: Seema Lekhwani**

Email: [s\\_lekhvani@yahoo.co.in](mailto:s_lekhvani@yahoo.co.in)

### Abstract

Blood transfusion has an important role in patients undergoing surgery. However, besides providing sufficient amounts of oxygen, blood transfusion should protect the safety of blood recipients too. Hemoglobin (Hb) is the most abundant intra-erythrocytic protein and reacts rapidly with nitric oxide (NO), the endothelium-derived relaxing factor that maintains blood flow and oxygen transport to tissues. The interaction of NO and Hb have been found to have unavoidable consequences. Clinical factors play an important role in the decision of transfusion if otherwise avoidable.

**Keywords:** Blood transfusion, Nitric oxide, Hemoglobin.

### Introduction

Although blood transfusion in patients going through surgical procedures can be revitalizing, still certain fears regarding associated morbidity and mortality.<sup>1</sup> The infusion of blood/blood products like; free hemoglobin (Hb), stored RBC supernatant and Hb-containing microvesicles oxidize nitric oxide (NO) as nitrate, thereby causing vasoconstriction, vascular dysfunction, and vascular injury.<sup>2</sup>

It is no less fascinating to note that on one hand, Nitric oxide (NO), an endogenous molecule, known for its vasodilatory and antiplatelet actions, produced in the endothelium causes vasodilation by increasing cyclic guanosine 3', 5'-monophosphate (cGMP) in the vascular smooth muscle;<sup>3</sup> and on the other, it is undoubtedly distressing to see that oxidized vascular NO as nitrate limits NO diffusion from endothelium to smooth muscle cells for activation of guanylyl cyclase; as a consequence, mean arterial blood pressure rises.<sup>4</sup> Moreover, the endothelial (constitutive) type III NO synthase, an isoenzyme, is an essential inducer of marrow cell mobilization thereby improving hemoglobin levels in blood because endogenous NO mediates erythropoietin activity and promotes the hypoxia inducible factor-1-DNA binding activity that leads to erythropoietin expression.<sup>5</sup> The interaction of NO and Hb have been found to have unavoidable consequences.<sup>6</sup>

### Aims and Objectives

Keeping the above theory in mind, the aim of the study was to evaluate the actual association between Hb and NO in patients who underwent surgery, in absence and deliverance of blood transfusion.

### Study and Design

This study was designed as a prospective analytical study which took approximately four months starting from December 2018 till March 2019 involving the Department of Blood Transfusion of B. P. S. Government Medical

College for Women, Sonapat and the Departments of Surgery and Biochemistry of Pt. B. D. Sharma PGIMS, Rohtak, Haryana, India.

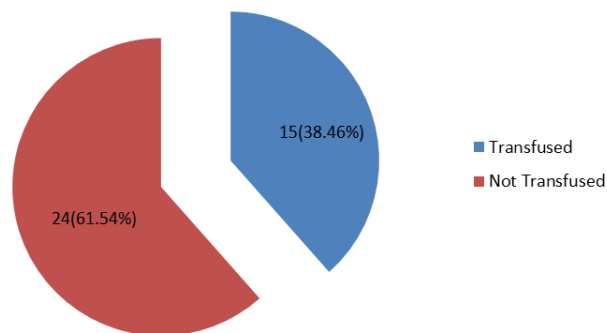
### Materials and Methods

The proposed study was approved by the Institutional Review Board and an informed consent was acquired from the patients who were found suitable for the study. Total thirty nine patients from the surgery ward were enrolled and those who received blood transfusion (n=15) were compared with those who did not receive blood transfusion (n=24) in terms of reduced hemoglobin (Hb) and nitric oxide (NO) levels. The Hb concentration in the whole blood was measured using automated hematology analyzer (Mindray BC-5800) based on the principle of modified hemoglobincyanide method spectrophotometrically. NO was measured in serum by Greiss reaction as nitrite (NO<sub>2</sub><sup>-</sup>) which is stable and non-volatile breakdown product of NO.

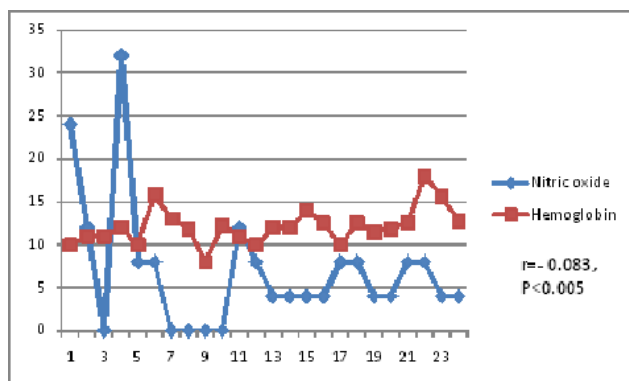
The differences between the two groups were evaluated using the 't-test' of significance and correlations were analyzed by Pearson's correlation.

### Results

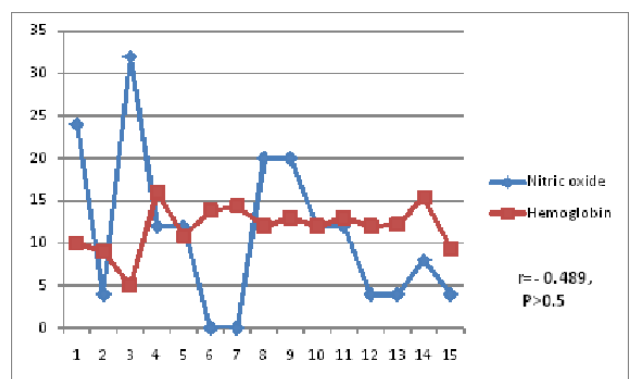
Those patients who did not receive any blood transfusion, a very weak but significant inverse correlation ( $r=-0.083$ ,  $P<0.005$ ) (Fig. 2) was observed between Hb and NO levels where as an insignificant but inverse correlation ( $r=-0.489$ ,  $P>0.5$ ) (Fig. 3) was noticed in patient who underwent transfusion of blood/blood product(s). Altogether, a significant and weak inverse correlation ( $r=-0.289$ ,  $P<0.05$ ) was found when all patients whether blood transfused or not were considered. Thereby, it seems to be an obvious feeble influence of the above two independent parameters upon each other in this study.



**Fig. 1:** Ratio between transfused and non-transfused (5:8) patients



**Fig. 2:** Weak, significant and inverse correlation between Hb and NO in non-transfused patients



**Fig. 3:** Insignificant inverse correlation between Hb and NO in non-transfused patients

## Discussion

Less than 40% of patients undergoing surgery received blood transfusion in this study (Fig. 1). The rationale behind providing blood transfusion has always been enhancing intravascular oxygen-carrying capacity for better tissue oxygenation<sup>7</sup>. However, recent studies have drawn attention also to the possible negative consequences of transfusion<sup>8-9</sup>. Enhanced susceptibility to oxidative damage of red blood cells and their microenvironment after transfusion, causes Hb release, a potent NO scavenger, thereby weakening vasodilatation and microcirculatory perfusion following transfusion<sup>8</sup>. Despite all the existing dilemmas, the overpowering helpful effects of transfusion cannot be

ignored. All the same, reports of the possible undesirable effects of transfusion on patient morbidity and mortality have increased in recent years.<sup>10-11</sup>

The major way that Hb destroys NO is through the dioxygenation reaction in which NO reacts with oxygenated hemoglobin to form methemoglobin and nitrate, so the ability of NO to diffuse from endothelial cells to smooth muscle cells to activate soluble guanylyl cyclase is limited but not totally hampered<sup>5</sup>. The NO dependent vasodilatation and enzyme action does indeed occur. NO produced in the endothelial cells by the enzyme NO synthase in red cell free zone created by blood flow velocity gradient, escapes scavenging. Nevertheless, red cell hemolysis results in release of free hemoglobin in the red cell free zone and enhances its scavenging potential<sup>12</sup>.

Though, we could not confirm a correlation between increased NO consumption and free hemoglobin as well as the duration of storage of blood in our study, in spite of this, it is interesting to note that an inverse correlation between the two parameters is present. It subtly indicates the existing threat of NO being scavenged by Hb though insignificant in those who were transfused blood<sup>13</sup>.

Our data may be of importance for those surgeries that do not require massive blood transfusion and are not an emergency but electively planned unrelated to heart or lung with good supportive care. Moreover, declining from transfusing an anemic patient might further worsen the scenario. The definite beneficial effects of blood transfusion should promote efforts for minimizing the harmful effects like ischemia and organ failure.

## Limitations

There being no facility to measure cell free hemoglobin in the Institute, its separate role in NO metabolism could not be elucidated despite our earnest efforts. Moreover, the duration of storage of blood could not be elucidated during sample collection procedure.

**Conflict of Interest:** None.

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