Impact of L-Arginine on Nitric Oxide Regulation in Pregnant Women Prone to Preeclampsia: Original Research

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ABSTRACT

Introduction: Preeclampsia is one of the major causes of maternal and perinatal mortality, especially in developing countries. Its etiology is still a matter of conundrum, but no specific cause has been identified. Evidence suggests that preeclampsia may be caused by endothelial dysfunction. Nitric oxide (NO), which is synthesized from L-arginine in endothelial cells by the endothelial nitric oxide synthase (eNOS), helps in vasodilation and helps in the adhesion of white blood cells and platelet aggregation. Alterations in the L-arginine-NO pathway are believed to be associated with the development of Preeclampsia. The main objective of the study is to evaluate the levels of nitric oxide and study the role of L-Arginine in decreasing the incidence of preeclampsia.

Material and methods: This study was three year interventional cohort study conducted at Osmania Medical College, Dr. VRK Womens College and hospital, Shadan medical college, Hyderabad. The ages of the cases varied between 18-35 years. The cases were grouped into 3 categories. All women in the Group 3 were given L-Arginine sachets of 3 gm twice a day until delivery and the level nitric oxide was analyzed clinically and statistically in all women at the 1st trimester of pregnancy (11-14 weeks) and between 17-22 weeks of pregnancy.

Result: There is a 37.7% chance of women developing preeclampsia if supplemented with L- Arginine and this data when compared to those who did not received L-Arginine had an 89.6% risk for developing Preeclampsia which is higher than the former data. Hence it can be stated that the incidence of Preeclampsia can be lowered by 51.9% by supplementing L-Arginine to those who are prone to develop Preeclampsia even before the disease could manifest.

Conclusion: This study shows that there was an increase in the blood levels of Nitric Oxide after L-Arginine was administered and the incidence of Preeclampsia was found to be lowered significantly in women who are prone to develop Preeclampsia.

Keywords: Preeclampsia; Nitric Oxide; Pregnancy; L-arginine; Hypertensive Syndrome

INTRODUCTION

Preeclampsia is defined as a hypertensive syndrome that occurs in pregnant women after 20 weeks' of gestation which complicates approximately 6-10% of all pregnancies and is the leading cause of fetal growth restriction, premature births, mortality and maternal complications.[1] Pre eclampsia is characterized by persistent increase in blood pressure (BP of ≥140 mmHg systolic and/or ≥90 mmHg diastolic, based on at least 2 measurements taken in 4 hours interval) with either proteinuria or systemic involvement.[2]

One consistent factor emerges that there is a compromised function of the maternal endothelium which contributes to the hemodynamic features of Preeclampsia.[3] The physiological basis for these changes is the development of intervillous spaces and trophoblast invasion of the myometrial portion of the spiral arteries so that the diameter of the vessels widens and more blood flow is shunted to the uteroplacental bed. Preeclampsia develops when there is poor invasion of the arteries and this is reflected by an early diastolic notch in the Dopplers of the uterine artery as early as 1st trimester of pregnancy.[4]

Recent studies indicate that Nitric Oxide which is a potent vasodilator plays an important role in relaxing smooth muscles and helps in the vasodilation of maternal blood flow. It also plays a significant role in fetoplacental blood flow and quiescence of the uterus before parturition. In some pregnant women, the concentrations of these nitric oxide levels can be low due to less bioavailability which may leads to the cause of preeclampsia. L-arginine is an amino acid present in the proteins of all life forms. It is classified as a semi-essential or conditionally essential amino acid. L-arginine, even when it is not an essential amino acid as defined above, is a vital one. In addition to participating in protein synthesis, it plays a number of other roles in the body. These include the detoxification of ammonia formed during the nitrogen catabolism of amino acids via the formation of urea. L-arginine acts as a precursor in the formation of nitric oxide. It is therefore logical that supplementation of L-Arginine would act as a Nitric Oxide donor in order to lower this incidence.[5]

Numerous *in vitro* experiments have shown that L-arginine has effects on endothelial cells that could be expected to

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inhibit cardiovascular disease. Inferences have been drawn from these studies suggesting that L-arginine, through its nitric oxide activity, especially in the endothelial cells of the blood vessels, inhibits vasoconstriction, thrombolytic activity, cell proliferation, inflammation and other activities that promote cardiovascular disease.[6]

The women who are prone to develop Preeclampsia are recognized by abnormal wave patterns in Doppler ultrasound between 17-20 weeks of pregnancy and even as early as the 1st Trimester (between 11-14 weeks) of pregnancy.[7] This is based on the principle that there is reduced uteroplacental perfusion as a result of abnormal trophoblast invasion of the spiral arterioles.[8] A Doppler study done in the 1st Trimester and between 17-20 weeks of gestation and blood estimates of these enzymes would help recruit women who are prone to develop preeclampsia in this study.[9] The use of Doppler studies is time tested and this modality is routinely used nowadays to predict woman who are likely to develop preeclampsia during their pregnancy.[10] From the present study, it is observed that doppler diagnosis is not the only method of screening for preeclampsia but there are several other screening tests like serum nitrate levels that could predict preeclampsia in woman.

It would also emerge from this study that how far the supplementation of L-Arginine, a nitric oxide donor would help in reducing the incidence of preeclampsia. This study would also open doors for better management of this elusive disorder of pregnancy by a way of supplementation of not only nitric oxide donors, calcium, low dose aspirin but also antioxidants for which larger and more extensive studies could be undertaken. In the present study we checked nitric oxide levels in pregnant women and investigated the role of L-Arginine in lowering the incidence of preeclampsia.

MATERIAL AND METHODS

This study was a three year interventional cohort study conducted at Osmania Medical College, Dr. VRK Womens College and hospital, Shadan medical college, Hyderabad. The ages of the cases varied between 18-35 years and older women were excluded from the study as age related factors can contribute to the development of hypertension in pregnancy. Only primigravidae were recruited for this study. All the patients those were diagnosed with the abnormal doppler were considered for further estimation of nitric oxide levels in the blood serum of first trimester pregnant women.

All cases were grouped into 3 categories, Group 1 (Normal Doppler), Group 2 (Abnormal Doppler with no L-Arginine supplementation), Group 3a (Abnormal Doppler with supplementation of L- Arginine at around the 17th week of pregnancy), Group 3b (Repeat blood biochemistry of Group 3a women who received L-Arginine). All women in the Group 3 were given L-Arginine sachets of 3 gms twice a day until delivery, in addition to their regular supplementation of Iron, Calcium, Folic acid and multivitamins (Women in Groups 1, 2, and 3 received regular supplementation of Iron Calcium and Folic acid). All the blood levels were repeated in Group 3 after the 32nd week of gestation to form a Group 3b in order to see whether there was any statistically significant improvement in the above blood levels more especially serum nitric oxide levels to know whether the nitric oxide levels were increased or not after the use of L-Arginine was administered. These levels were analyzed in all women at the 1st Trimester of pregnancy (11-14 weeks) and between 17-22 weeks. In all the cases placenta was sent for histopathology.

Ethical Approval: The protocol was first sent to the Ethical Committee of Osmania Medical College and approval was obtained as the study involved administering a drug. A written informed consent was taken from pregnant women in their own language prior to enrollment of the cases into the study.

Nitric Oxide Estimation

Doppler Ultrasound of the Uterine Arteries was analyzed in all women. To the collected serum Griess reagent (1% sulfanilamide and 0.1% *n*-(1-naphthyl) ethylenediamine dihydrochloride in 5% H_3PO_4; Sigma, St. Louis, MO) was added and incubated for 10 mins at room temperature. Read absorbance with a spectrophotometer at 570 nm.

STATISTICAL ANALYSIS

Descriptive statistics were calculated for all variables. Mean values were compared across groups by ANOVA with post hoc test LSD/t test. Pre and post values were compared by paired 't' test. To study the associations with categorical variables, chi square test is used. Level of significance is considered as 0.05. SPSS version 19.0 is used.

RESULTS

The main agenda of estimating serum nitric oxide levels in the present study revealed that very low levels of 'NO' i.e. 0.76 umol/L and 0.52 umol/L in women who were prone to develop Preeclampsia (Group 2 and 3a). Whereas the 'NO' levels in normal group (Group1) was shown with 2.00 umol/L. The patients of Group 3b; who were supplemented with L-Arginine showed a mean of 2.15 umol/L which is statistically significant with the *p*-value of < 0.001.1. In Group 1 (Normal Dopplers) when the NO levels were <2 umol/L (17 cases) 41.2% developed Hypertension (Table 1a and b) and when the levels were >2 umol/L (30 cases) no

Group No	No. of women	Mean Value (umol/L)	Std. deviation	Std. error	*p*-value
1	47	2.0045	1.18303	0.17256	< 0.001
2	50	0.7690	0.65478	0.09260	< 0.001
3A	83	0.5298	0.36077	0.03960	< 0.001
3B	60	2.1502	0.7426	0.09557	< 0.001

Table-1a: Comparison between normal and preeclamptic women with normal doppler.

Crosstab[a]					
			Hypertension		Total
			Normal	HTN	
Nog	<2	Count	10	7	17
		% within nog	58.8%	41.2%	100.0%
	>=2	Count	30	0	30
		% within nog	100.0%	0.0%	100.0%
Total		Count	40	7	47
		% within nog	85.1%	14.9%	100.0%

a. group = 1

Table-1b: Group 1 normal dopplers patients with the hypertension.

Crosstabulation					
			nog		Total
			<2	>=2	
grp	1	Count	17	30	47
		% within grp	36.2%	63.8%	100.0%
	2	Count	48	2	50
		% within grp	96.0%	4.0%	100.0%
	3	Count	82	1	83
		% within grp	98.8%	1.2%	100.0%
Total		Count	147	33	180
		% within grp	81.7%	18.3%	100.0%

Table-2: Before supplementation of L- Arginine in Group 1.

nog * nofg Crosstabulation					
			Nofg		Total
			<2	>=2	
Nog	<2	Count	21	38	59
		% within nog	35.6%	64.4%	100.0%
	>=2	Count	0	1	1
		% within nog	.0%	100.0%	100.0%
Total		Count	21	39	60

Table-3: After supplementation of L-Arginine in Group 1

Crosstab[a]					
			Hypertension		Total
			Normal	HTN	
Nog	<2	Count	5	43	48
		% within nog	10.4%	89.6%	100.0%
	>=2	Count	1	1	2
		% within nog	50.0%	50.0%	100.0%
Total		Count	6	44	50
		% within nog	12.0%	88.0%	100.0%

Table-4a: Group 2- Comparison between normal and preeclamptic women without L- Arginine supplementation.

patient developed Hypertension. Before supplementation, 63.8% cases showed normal values of >2 umol/l in Group 1 (Table 2) whereas only 4% and 1.2% cases showed values > 2 umol/l in Groups 2 and 3 respectively. After drug administration 64.4% cases showed with elevated levels of 'NO' (>2 umol/l (Table 3) which implies that L-Arginine does play an important role in increasing the levels of Nitric oxide in the blood.

Preeclampsia as compared to those who have not received

Crosstab[a]					
			Hypertension		Total
			Normal	HTN	
nog	<2	Count	43	26	69
		% within nog	62.3%	37.7%	100.0%
	>=2	Count	1	0	1
		% within nog	100.0%	.0%	100.0%
Total		Count	44	26	70
		% within nog	62.9%	37.1%	100.0%

Table-4b: Results of Group 3 with supplementation.

Crosstab					
			Hypertension		Total
			Normal	HTN	
Nog	<2	Count	58	76	134
		% within nog	43.3%	56.7%	100.0%
	>=2	Count	32	1	33
		% within nog	97.0%	3.0%	100.0%
Total		Count	90	77	167
		% within nog	53.9%	46.1%	100.0%

Table-5: Comparison between normal and preeclamptic women before and after supplementation of L-Arginine.

L-Arginine (Group 2) has 89.6% risk for developing Preeclampsia (Table 4a). It can therefore say that the incidence of Preeclampsia can be lowered by 51.9% by giving L-Arginine to those who are prone to develop Preeclampsia even before the disease is manifested. L-Arginine which is a Nitric Oxide donor if supplemented in those who are in Group 3 have a 37.7% (Table 4b) chance for developing preeclampsia was reduced to zero percentage.

In case of less 'NO' levels i.e. <2 umol/L, 56.7% developed Hypertension (Table 5) and when the 'NO' levels were > 2 umol/L only 3% of the patients developed Hypertension**.** In Group 3, out of 70 cases only 1 had a 'NO' value of > 2 umol/L who did not develop Hypertension and the remaining 69 cases had 'NO' value of < 2 umol/L, 37.7% cases had hypertension. It can therefore be stated categorically that the levels of 'NO' in the blood has a statistical significant correlation between the development of hypertension and with 'NO' values are less than 2 umol/L. The predictive indices for 'NO' for the development of hypertension have a sensitivity of 95.8% and a specificity of 85.1%. The positive predictive value was 89.6% (if the value is abnormal i.e < 2umol/L) and the negative predictive value was 96.1% (if the value is normal i.e > 2umol/L).

The histopathological results revealed that the placenta was normal except in 20 cases which showed infarcts and calcification and in these 20 cases 18 preeclampsia women who did not receive L-Arginine and 2 cases with preeclampsia who received L-Arginine.

DISCUSSION

The role of nitric oxide in the pathogenesis of Preeclampsia was studied by Seligman SP *et al*[11] concluded that the circulating levels of nitrite are decreased in women with preeclampsia. The present study also concurs with the work

done. The possible explanation is a compromised function of maternal endothelium that contributes to the hemodynamic features of preeclampsia, i.e. vasoconstriction with increased peripheral resistance and hypertension [12]. Isolated small arteries from women with preeclampsia demonstrate impaired endothelium dependent vasodilatation. Nitric oxide a potent endothelial-derived vasodilator synthesized by constitutive nitric oxide synthase (NOS-3) from L-arginine, was shown to modulate peripheral vascular tone.[13] The findings in the by Facchinetti F, *et al.*[14] study partially supports the hypothesis that preeclampsia is characterized by a dysfunction of the L-Arginine – Nitric Oxide pathway, which provides a base of the present study. Our study could be compared with a study conducted by Rytlewski K, et al.[15] who concluded that L-Arginine supplementation decreased blood pressure in women with preeclampsia through increased endothelial synthesis and/or bioavailability of nitric oxide. In this present study there is a correlation between the development of Preeclampsia and the levels of plasma nitric oxide levels which shows its significance in lowering preeclampsia. Also, a step further analysis of 50 women who were apparently normal pregnancy with normal Dopplers and having majority of them had normal Nitric oxide levels, followed their pregnancy outcome, development of preeclampsia and they were not supplemented with L-Arginine. The results of the present study is substantiated by a study conducted by Hudicek- Martincic G *et al*.[16] on Nitric Oxide during pregnancy and delivery suggested that higher levels of Nitric oxide are utilized in pathological conditions of pregnancy like IUGR preeclampsia and preterm labour. Another case from Khetsuriani T *et al*.[17] showed that during pregnancy the free Nitric oxide content was decreased by 10% and this was in unison with the present study too. The study conducted by Buhimschi IA *et al*,[18] where the nitric oxide pathway in preeclampsia was studied on rats who were infused NG-nitro-L-Arginine (L-NAME) a nitric oxide synthase inhibitor which is used as an animal model for preeclampsia and the conclusion drawn from their work is that preeclampsia is a state of nitric oxide deficiency which is concurrent with our present study. Contrary results were seen in a study done by Hladunewich MA *et al*[19] where the Effect of L-Arginine therapy on Preeclampsia was observed. Despite a significant increase in postpartum serum Arginine levels, there were no significant blood pressures differences in both groups. A study based on the assumption that agents which increase nitric oxide may prevent Preeclampsia was conducted by Meher S *et al.*[20] They concluded that there was insufficient data for reliable conclusions. Another paper published by Lowe DT *et al.*[13] reviewed the evidence that the primary dysfunction in preeclampsia is a relative dysfunction of available nitric oxide which correlates with our present study. This study also points to further scope of research in this field wherein pregnant women who are prone to develop Preeclampsia could be further evaluated with other nitric oxide donors like Isosorbide Dinitrate sublingually or Glyceryl trinitrate dermal patch. It also emerges that nitric oxide as said by several authors is indeed the molecule of the decade.

CONCLUSION

It is concluded that there was statistical significance between L-Arginine supplementation and Nitric Oxide levels in women with preeclampsia. The study also shows that there was an increase in the blood levels of nitric oxide after L-Arginine supplementation and these women had lesser chances of developing preeclampsia compared to those who did not receive the L-Arginine supplementation. This study suggests the specificity of nitric oxide as a marker for the development of preeclampsia.

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