

Role of lactoferrin and methyldopa combination in management of hypertension with pregnancy

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Abstract

In this research paper, 60 women suffering from Hypertensive disorders with pregnancy divide into three groups group (A) 20 cases treated with lactoferrin, group (B) 20 cases treated with lactoferrin and methyldopa, and group (c) 20 cases treated with methyldopa. The blood pressure was measured at first visit then every weeks. General obstetric examination and ultrasound and biochemical investigation were done in all cases. Results clearly shows decrease in both systolic and diastolic blood pressure were recorded after one month treatment with significant decrease in both systolic and diastolic blood pressure in all groups. But group B tight control of blood pressure other benefit of lactoferrin as correction of anemia. Lactoferrin acceptable, tolerated and minimal side effect and adverse effects of methyldopa decrease by combination with lactoferrin. This research paper concludes that the combination of lactoferrin with methyldopa was efficacy and safety in control hypertension with pregnancy by its antihypertensive effect of lactoferrin and its other benefit antioxidation, immunomodulatory and correction of anemia.

Keywords: hypertension with pregnancy, lactoferrin, methyldopa

1. INTRODUCTION

Hypertension in pregnancy remains a significant public problem with significant maternal and fetal morbidity and mortality¹ and no effective treatment for this disease, and delivery of placenta remain the only solution. Hypertensive disorders in pregnancy include pre-eclampsia, gestational hypertension and chronic hypertension². The pathogenesis of pre-eclampsia is still unclear factors such as oxidative stress, circulating placenta-derived factors, immunologic defects, and genetic factor may all be important

³as regarded anti-angiogenic factors are known to role in path physiology of pre-eclampsia⁴ two antiangiogenic factors increase in pre-eclampsia soluble FM's -like kinase 1 (sFlt1)

and soluble endoglin (sEng)but vascular endothelial growth factor(VEGF) and placental growth factor (PlGF) are decrease some of these changes can be detected several weeks before appearance of clinical symptom of preeclampsia⁵vascular endothelial dysfunction characterized by vasoconstriction and low anticoagulant activity⁽⁵⁾. Reactive oxygen species (ROS) seem to play a critical role in the Pre-eclampsia. Oxidative stress can induce the adhesion of leukocytes and platelets to the endothelium as well as the release of cytokines and anti-angiogenic factors. Adhesion of blood cells and endothelial cells is critical in the inflammation process involved in the pathogenesis of pre-eclampsia As a result of the inflammation, generalized vasoconstriction and increased resistance in the placental circulation can be caused by reduced utero placental blood flow followed by placental dysfunction⁶. The interaction between NO and ROS modulates vascular tone. Thus the altered balance of NO and ROS also seem to play a critical role in the pathogenesis of PE⁷ suggest that pre-eclampsia can be resolved by removal of the placenta because this organ is a major source of NO and ROS⁷. Pre-eclampsia placenta is known to be hypoxic and stimulate the release of a large amount of syncytiotrophoblast microparticles⁸. Which stimulate the production of damage-associated molecular pattern which activates immunocytes including neutrophils and dendritic cells. Activated immunocytes could produce pro inflammatory cytokines including tumor necrosis factor (TNF α) and promote oxidative stress through neutrophil nicotinamide adenine dinucleotide phosphate oxides' activation. The increased advanced glycation end products interacts with the receptors (RAGE) in pre-eclampsia and activates NADPH oxidase RAGE/NADPH oxidase-dependent pathway promotes sFlt-1 expression in trophoblasts and is involved in the increased oxidative stress in pre-eclampsia placenta⁹. Methyldopa has been assigned as a category B drug by the US Food and Drug Administration and is frequently used for the treatment of PIH. Methyldopa acts centrally by decreasing sympathetic tone, and its mechanism of action of depends on its metabolism in the liver and intestines to α -methyl norepinephrine, which acts as a "false neurotransmitter." By competitively inhibiting the sympathetic effects of norepinephrine, it decreases systemic arterial pressures and also decreases plasma renin activity¹⁰.

Alpha methyl dopa acts on α_2 -adrenergic receptors, primarily in the central nervous system (CNS) although an effect on peripheral α_2 -adrenoreceptors may also play a part¹⁰ α_2 -adrenoreceptors have also been identified in a variety of other human tissues outside the CNS, including myometrium and placenta¹¹. An almost universal effect of α_2 -adrenoreceptor stimulation is the inhibition of adenylyl cyclase which leads to decreased production of cAMP¹², cAMP has been shown to be a strong inducer of Flt-1 expression in mice^{13, 14}. Methyl dopa reduced sFlt-1 concentration in culture medium, increased VEGF concentration¹⁵. Other study of Podjarny et al., The efficient action of the sympathetic antagonist methyl dopa may be due not only to its antihypertensive effects but also by its stimulating effect on NO synthesis leading also to an improvement of renal function¹⁶ and does not seem to have no adverse effects on uteroplacental or fetal hemodynamic¹⁷. Adverse effects as sense of fatigue, depression

In some patients and decreased salivation leading to xerostomia. Methyl dopa can also cause elevated liver enzymes in 5%, hepatitis and hepatic necrosis have also been reported. Some patients will develop a positive antinuclear antigen or ant globulin (Coombs') test with chronic use, and this is occasionally clinical hemolytic anemia^{18, 19}. Lactoferrin (LF), a natural iron-binding protein in milk, LF is a single-chain glycoprotein with a molecular mass of ~80 kDa that belongs to the family of transferrin²⁰, is widely distributed in several secretion especially milk and also, neutrophilic granules content²¹. Lactoferrin has been assigned with multiple biological function including considering the first-line defense protein involved in protection against a multitude of microbial infections²², anti-viral²³, anti-parasitic^{24, 25}, anti-fungal²⁶, immunomodulation activity, cell growth regulation function anti-inflammation and antinociception^{27, 28}. LF and anticancer activities. Lactoferrin gene expression to the potential use of lactoferrin in cancer therapy. Lactoferrin cytotoxicity against several cancers is reported to occur in distinct ways under different conditions, namely by cell membrane disruption, apoptosis induction, cell cycle arrest, and cell immunoreaction²⁹. LF receptors are found in lymphocytes, platelets, macrophages, dopaminergic neurons, megakaryocytes, and endothelial cells. Some of these receptors are involved in LF uptake. In the cerebral endothelial cells, LF is transported through receptor-mediated processes without any intra endothelial degradation³⁰, anti oxidative mechanisms³¹, Sequestration of iron by lactoferrin reduces insult-induced oxidative stress³². Lactoferrin is exerting changes on leukocytes of the innate immune system, through increasing natural killer (NK) cell activity³³. Lactoferrin and hypertension Safaeian L et al antihypertensive studied effect of LF in dexmethasone-induce hypertension due to inhibition of ACE and ECE and endothelial-depend relaxant action NO synthase inhibitors, with its vasodilators action³⁴, also, Mladenka et al., studied the effect of lactoferrin in rat model of catecholamine, and concluded that

LF reduced impairment caused by isoprenaline as stroke volume decrease and increase peripheral resistance and calcium overload. These positive effect were likely to mediated by positive inotropic effect of lactoferrin and antioxidation by inhibition of ROS formation due to chelation free iron³⁵, while, Ikeda and coworkers have reported significant phosphorylation of Src, Akt, and endothelial nitric oxide synthases' (eNOS) after treatment with LF suggesting a Src Akt eNOS-dependent pathway in promotion of vascular endothelial cell function by LF³⁶. Add to that Hayashida K. studied hypotensive effect of LF was cause via an endothelium-dependent vasodilatation and strongly mediated by the central opiodergic system³⁷.

The aim of this study was to evaluated antihypertensive action of lactoferrin in hypertension disorder with pregnancy added to its other benefit as anti-oxidation, immune modulator, iron homeostasis, anti-infection, and anti-inflammatory and studies its effectiveness safety and acceptability and studies its action in combination with methyl dopa hoping to increase effectiveness and decrease side effect of methyl dopa.

2. Methods

This study conducted in department of Obstetrics and Gynecology at Damietta hospital (outpatient). After taking written informed consent from pregnant women prior commencing. A total of 60 pregnant women were enrolled and randomly assigned into three study groups. Group a (lactoferrin only) Included 20 pregnant received lactoferrin 100mg 3 times daily. Group B (lactoferrin and methyl dopa) Included 20 pregnant received lactoferrin 100mg 3 time and methyl dopa 250 3 time daily. Group C (methyl dopa only) included 20 pregnant received methyl dopa 250 3 time daily. Pregnant women were diagnosed as hypertension with pregnancy systolic blood pressure 140 or more diastolic 90 or more. All case histories were taken included personal obstetric family history. Examination: general, obstetric were taken, fetal wellbeing by ultrasound and Doppler, blood pressure pre and post treatment systolic and diastolic BP of all groups was monitored in first day and in seven day every week for one month and comparison between pretreatment and after one month of treatment. Reprobated any complication of pregnancy to mother fetal and any possible side effect of treatment to mother or to fetus related to any of drug or its combination as gastrointestinal discomfort, headache, vertigo, postural hypotension, bradycardia, sedation, myalgia, arthralgia and agranulocytosis. Maternal acceptability with treatment (poor compliance, over all satisfaction with treatment were recorded.

3. Laboratory Methods

Complete blood picture, urine analysis, liver function and renal function tests were done.

Statistical Analysis were performed by using statistical software SPSS version "17" categorical variations were compared using mean, standard deviation(SD), student t-test. Statistical significance was defined as P value < 0.05.

4. Statistical analysis

5. Result

Figure (1): Systolic blood pressure (Mean±SD)

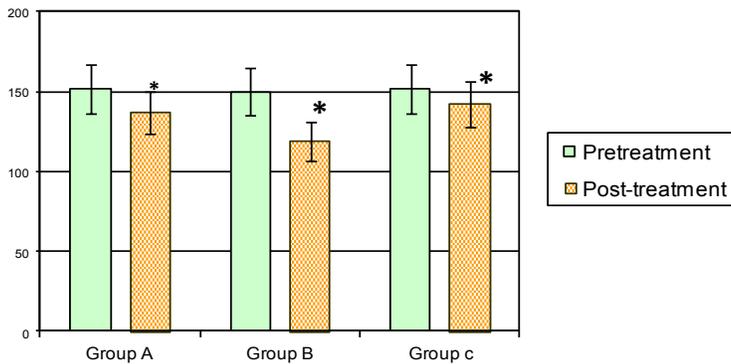


Figure (2): Diastolic blood pressure (Mean±SD)

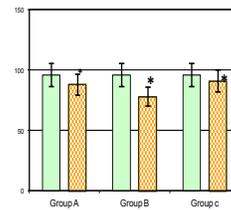


Table 1. Mean ± standard deviation systolic blood pressure Pretreatment and post treatment after one month

Group	pretreatment		post treatment		T test	P value
	systolic pressure	blood	systolic pressure	blood		
A	152,75 ± 6.6		137 ± 8		2.36E-08	<0.05
B	150.25 ± 6.8		119 ± 4.5		7.91E-20	<0.05
C	152.5 ± 5.7		142.5 ± 6.97		7.73E-06	<0.05

Table 2. .Mean ± standard deviation diastolic blood pressure Pretreatment and post treatment after one month

Group	pretreatment diastolic blood pressure	post treatment diastolic blood pressure	T test	P value
A	96 ± 3.84	88.5 ± 2.86	1.19E-08	<0.05
B	96.25 ± 4.25	78 ± 3.4	7.81E-18	<0.05
C	96 ± 3.84	91.5 ± 5.15	0.001673	<0.05

Table 3. Maternal & Fetal complication

	A	%	B	%	C	%
Sever preeclampsia	0	0	0	0	1	0.05
Preterm labor	0	0	0	0	2	10
Urinary tract infection	0	0	0	0	3	15
IUGR	0	0	0	0	2	10
Olighydraminosis	0	0	0	0	2	10
Anemia	0	0	0	0	7	35

Table 4.Side effect of treatment

	A	%	B	%	C	%
Neasue	2	1	2	10	2	10
Vomiting	1	5	1	5	2	10
Headache	0	0	0	0	7	35
Drowsiness	0	0	4	20	8	40
Bradycardia	0	0	0	0	2	10
Weakness	0	0	4	20	8	40
Myalgia	0	0	4	20	7	35

Table 5. Maternal acceptability with treatment

Group	A	%	B	%	C	%
Poor compliance	0	0	1	5%	5	25
Satification with	20	100%	9	95%	15	75

In table (1) and figure (1): Mean systolic blood pressure Pretreatment and post treatment after one month. In group A, the mean systolic before treatment was mmHg 152.75 ± 6.6 mmHg and after treatment reduced to 137 ± 8 mmHg. The reduction in systolic blood pressure was statistically significant compared to pretreatment, while in group B, the mean systolic before treatment was 150.25 ± 6.8 mmHg and after treatment reduced to 119 ± 4.5 mmHg. The reduction in systolic blood pressure was statistically significant compared to pretreatment. In group C, the mean systolic before treatment was 152.5 ± 5.7 mmHg and after treatment reduced to 142.5 ± 6.97 mmHg. The reduction in systolic blood pressure was statistically Significant compared to pretreatment.

In table (2) and figure (2): Mean diastolic blood pressure Pretreatment and post treatment after one month. In group A, the mean diastolic before treatment was 96 ± 3.84 mmHg and after treatment reduced to 88.5 ± 2.86 mmHg. The reduction in diastolic blood pressure was statistically significant compared to pretreatment, while in group B, the mean diastolic before treatment was 96.25 ± 4.25 mmHg and after treatment reduced to 78 ± 3.4 mmHg. The reduction in diastolic blood pressure was statistically significant compared to pretreatment and in group C; the mean diastolic before treatment was 96 ± 3.84 mmHg and after treatment reduced to 91.5 ± 5.15 mmHg. The reduction in diastolic blood pressure was statistically significant compared to pretreatment.

Table (3): Maternal and fetal complication. In group C, three cases progressive to Sever pre-eclampsia and we increase dose of methyldopa to 500 3time per daily and admitted to hospital one cases progressive eclampsia. Two cases diagnosed preterm labor and three cases diagnosed urinary tract infection and while iron deficiency anemia were diagnosed in seven cases as regarded to Fetal complication in group C as intrauterine growth retardation and oligo-hydraminos were recorded, but in group B, no recorded complication and even decrease dose of methyldopa 250 twice daily and other benefit of treated of anemia. In group A, no recorded cases of anemia but five cases not responded to lactoferrin alone and uncontrolled blood pressure and added to it antihypertensive drug in two cases and no recorded fetal complication.

Table (4): Side effect of treatment. In group C, methyldopa alone with complication more than group A or B, as weakness, drowsiness, myalgia and bradycardia. In Group a, minimal side effect in the form of gatero-intstinal symptom as nausea and vomiting. In group B, side effect severity less than group C, this means lactoferrin may modality side effect of methyldopa.

In Table (5): In group lactoferrin 100% acceptability with no compliance. In group B, combination of both drug 95% acceptability and 5% poor compliance. In group C, methyldopa acceptability 75% and 15% poor compliance. Lactoferrin acceptable than methyldopa and Better tolerated alone or with methyldopa.

6. DISCUSSION

Lactoferrin as a food-derived peptide is believed to be safer for currently used for hypertension treatment. Our study showed that decrease in systolic and diastolic blood pressure in group A, group B and group C but in group B tight control than C blood pressure reach to normal value in both systolic and diastolic and by the combination of treatment blood pressure controlled to normal value with no need to increase dose of methyldopa in opposite to group B in which dose of methyldopa increase up to 500mg 3 time per day in many cases or need to combination of methyldopa with other drugs as beta blocker to control blood pressure, so our study showed that lactoferrin benefit as antihypertensive but in its benefits augmented by combination with methyldopa which in other side play role in modulated action of methyldopa by increase its advantage action and in decrease its adverse effect more benefits as antihypertensive for both action of antihypertensive and hypertensive effect of methyldopa The result of our study that lactoferrin alone act as antihypertensive but its effectiveness increase and more potent response recorded in combination with methyldopa and various studies have shown similar finding regarding antihypertensive action of lactoferrin which act by many mechanisms.

The first mechanism may be attributed to the angiotensin converting enzyme and endothelin converting enzyme inhibitor

action and also endothelium-dependent relaxant action of LF as in following studies of Hayashida K et al³⁷, and also, Ricard F and coworker, have reported that low molecular weight bovine lactoferrin less than 3 kDg has potential application as nutraceutical for hypertension treatment as reduced angiotensin I converting enzyme, angiotensin II- and aldosterone but increase renin³⁸. In study of Paloma Manzanare, antihypertensive effect of lactoferrin act by inhibits angiotensin I-converting enzyme modifies expression of hypertension-related genes enhances nitric oxide production in cultured human endothelial cells³⁹, Safaeian L et.al study, antihypertensive effect of LF in dexmethasone-induce hypertension and mention that LF had antihypertensive effect due to inhibition of ACE, ECE, endothelial-depend relaxant action and NO synthase inhibitors with its vasodilators action³⁴. The second mechanism responsible for antihypertensive effects of LF in study of Ikeda and coworkers, had reported vasodilator action of LF is strongly mediated by NO production because of complete blockage of this effect by a NO synthase inhibitor and also have reported significant phosphorylation of Src, Akt, and endothelial nitric oxide synthase (eNOS) after treatment with LF suggesting a Src Akt eNOS-dependent pathway in promotion of vascular endothelial cell function by LF³⁶, whereas another study by Ruiz-Giménez, had reported the vasodilator action of LF is mediated by NO production because of complete blockage of this effect by a NO synthase inhibitor⁴⁰, the study of Ken-ichiro and coworker mention that bovine lactoferrin has antioxidant dependent hypotensive effect in rat and suggested that lactoferrin effect cause via an endothelium depend vasodilatation that is strongly mediated by NO production also, by central opiodergic system³⁷. The third mechanism responsible for antihypertensive effects of LF antioxidant effect, Mladenka P et al., studied the effect of lactoferrin in rat model of catecholamine and concluded that LF reduced impairment caused by isoprenaline as stroke volume decrease, an increase peripheral resistance and calcium overload. These positive effect were likely to mediate by positive inotropic effect of lactoferrin and antioxidation by inhibition of ROS formation due to chelation of free iron³⁵. The present study had shown that lactoferrin and methyldopa combination with less maternal and fetal complication than methyldopa alone in control hypertension we recorded decrease incidence of preterm labor and less percentage of cases diagnosed as urinary tract infection and the benefit recorded in study group in women treated with Lactoferrin correction of iron deficiency anemia. This agree with study of Mohamed Rez et al., his study showed that lactoferrin was better and more acceptable with higher increase in hemoglobin compared to oral iron over two month can used as good substitute to oral iron in mild to moderate anemia⁴¹, also in the study of Paesano, showed that bovine lactoferrin improved hematological parameters as total serum iron and serum ferritin compared to treatment with ferrous sulfate⁴². Side effect of lactoferrin minimal in form of nausea and vomiting were recorded in few cases in contrast to methyldopa group

which had many side effects this agree with study of Ismail A⁴³, in his experimental results revealed that methyldopa slightly increased Malondialdehyde and Myeloperoxidase levels in rat kidney tissue slightly reduced total glutathione so the combination of treatment in group B side effect of methyldopa decrease may be due to antioxidation effect and immunomodulation this is also, agree with study of Kimoto Y⁴⁴ et al., rerecorded the protective effect of lactoferrin on Cisplatin-induced nephrotoxicity in rats by induce diuresis and nitric oxide dependent relaxation of smooth muscle.

Lactoferrin is acceptable than methyldopa and better tolerated alone or with methyldopa this is agree with result of Peason et al, He mention that Lactoferrin is used in pregnancy as it natural drug without adverse effects it well tolerated and has excellent safety profile with no adverse effect⁴⁵

Therefore Lactoferrin in combination with methyldopa can be used in management of hypertension with pregnancy by its antihypertensive action and by modulated function of methyldopa by augmented its advantage as antihypertensive action of and decrease its disadvantage action added to other benefit of lactoferrin as antioxidant effect, immunomodulatory action which promote angiogenesis and its action on iron metabolism may change in pathogenesis of pre-eclampsia and its prognosis. Further studies are need to obvious other benefits of lactoferrin as immunomodulatory, antioxidation, anti-infective and its role in control of pre-eclampsia.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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