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IMPACT OF READY TO USE FOOD SUPPLEMENTS (RUFS) ON THE RATIO OF SOLUBLE FMS LIKE TYROSINE KINASE-1 (SFLT-1) TO PLACENTAL GROWTH FACTOR IN PRE-ECLAMPTIC WOMEN FOR SIX MONTHS

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ABSTRACT

Purpose: Pre-eclampsia (PE) defined as an increased hypertension, proteinuria and edema following 20 weeks of gestation. It depends on the angiogenic biomarkers with the normal ratio (sFLT-1 / PlGF) less than 38, abnormality in the levels is positively correlated with the severity of preeclampsia and undesirable fetal (preterm delivery) and maternal outcome much before appearing the clinical signs and symptoms of the disease. Nutrients such as enriched with HDL, calcium, iron, iodine, copper, manganese and Omega-3 unsaturated fats play an important role in decreasing the danger of the pre-eclampsia. **Objectives:** In our study, we want to identify the correlation of nutritional lipid-based supplements (LNS) on the impact of angiogenic factors that are associated with pre-eclampsia. **Study Design:** Randomized contral trial. **Setting:** Saidu group of teaching Hospital Swat. **Period:** Jan to Jun 2018. **Material & Methods:** A total of eighty-four *(n=84)* pre-eclamptic participants with positive test of proteinuria (pre-selection) were recruited for our study sFLT-1 &PlGF levels was measured with commercially available ELISA kit (CatLog). **Result:** Mean concentration of control before [sFLT-1, 68±215-PlGF, 107±63 pg/ml] & after [sFLT-1, 161±322-PlGF, 104±57 pg/ml]. While interventional before [sFLT-1, 57±207-PlGF, 97±54 pg/ml] & after [sFLT-1, 184±866-PlGF, 107±113 pg/ml]. Weight and BMI was strongly significant. Ratio (sFLT-1/ PlGF) was positive in after intervention between control and intervention arm. **Conclusion:** In conclusion, Cut-offs score with the changes in sFlt-1/PlGF ratio may clinically utilize these markers for PE pregnancies with or without intervention (LNS). sFlt-1/PlGF proportion is more critical among control and intervention with an unaltered mean PlGF level in control pre-eclamptic patients. We infer that the analytic window in the utilization of the sFlt-1/PlGF ratio differs with LNS supplementation.

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INTRODUCTION

Preeclampsia, the multi system disorder is defined as the new-onset hypertension with the presence of proteinuria after 20 weeks of gestation in previous non-protein uric and normotensive pregnant women.[1] The serious complications of preeclampsia include eclampsia, increased levels of liver enzymes, hemolysis, decreased platelet count (HELLP) syndrome,[2] fetal retardation, underweight newborn, preterm delivery, fetal death and newborn deaths.[3] The Global incidence of preeclampsia is about 3.2% of total births, estimated more than 4 million cases per year and more than about 72,000 were reported fatalities.[4] In Pakistan hypertension in pregnancy is responsible for 30% of total maternal mortality and preeclampsia is a major cause which complicates 5-10% of pregnancies.[5]

In spite of the large number of researches, the pathophysiology of preeclampsia remains largely

unknown. The mechanism by which the risk of future cardiovascular diseases increases in the hypertensive disorders of pregnancy may be associated with the presence of biomarkers related to cardiovascular disease after delivery even after the declaration of elevated blood pressure.[6] The abnormal circulating angiogenic factors reported pathogenic role in preeclampsia.[7] Studies reported various factors in pathogenesis of preeclampsia including soluble FMS- like tyrosine kinase 1 (sFLT-1) and the placental growth factor (PlGF).[8] sFLT-1 (soluble FMS- like tyrosine kinase 1, also known as sVEGFR-1) is a secreted protein, a splice variant of vascular endothelial growth factor (VEGF) receptor, sFLT-1 lacking the trans-membrane cytoplasmic domain of the membrane-bound receptor.[9] In preeclampsia sFLT-1 is markedly increased, this increased level of sFLT-1 is strongly associated with decreased levels PlGF, further confirmed by different studies.[10,11] The normal ratio (sFLT-1 / PlGF) must be less than 38, abnormality in the levels of these angiogenic factors is correlated with the severity of preeclampsia and undesirable fetal (preterm delivery) and maternal outcome much before appearing the clinical signs and symptoms of the disease.[12] These angiogenic factors can contribute in differentiating preeclampsia from other disorders of pregnancy presenting same clinical profiles.[13]

New researches suggested that lipid nutrient supplements can reduce both stunting and wasting in newborns.[14] In lipids, various investigations of biomarkers, reported dyslipidemia in preeclampsia, such are decreased HDL, higher triacylglycerols and LDL cholesterol.[15] One investigation shows the difference in unsaturated fats and triacylglycerols that were present before 20 weeks of gestation[16] but there is no obvious proof that these differences were associated with daily individual dietary consumption. Omega-3 unsaturated fats, have been proposed to be essential in the inhibition of preeclampsia. The likelihood of the gainful impact of these unsaturated fats was seen in population ingesting fish oil having less prevalence of preeclampsia. In a study with more than 5000 pregnant women were appointed to get supplementation (included calcium, iron, iodine, copper and manganese) or placebo from 20 weeks of gestation. A 31 % decrease in the rate of preeclampsia was observed with strong positive statistics ($p=0.005$).[17] Lower calcium levels have been best concentrated in few epidemiological studies to their relationship in preeclampsia.[18] These perceptions prompted the speculation that the frequency of preeclampsia can be decreased by the administration of calcium supplementation.[19] A Cochrane survey in 6894 ladies demonstrated a 32% decrease of the frequency of preeclampsia with calcium supplementation.[20] A relationship between zinc and preeclampsia has been recommended by decreased zinc plasma levels in ladies with preeclampsia.[21] However, to adjust the return of preeclampsia with zinc supplementation has not been effective.[21,22]

The achievements are known about the effect of magnesium in the treatment for eclamptic seizures *in-vitro.*[23] Further, proposed that magnesium may be present in inadequate concentration with preeclampsia. Besides, prospective study of the magnesium serum levels was not strong with FAQs and dietary intake of 30 weeks of gestation in pre-eclamptic women.[24] During preeclampsia iron status have been accounted for their impaired levels.[25] A few investigations proposed a relationship with low hemoglobin levels[26] and decreased transferrin with women having preeclampsia. Likewise, low levels of iron should be interpreted with cautions as the decreased ratio of transferrin or increased concentration of ferritin is also strongly associated with inflammation, which in turn link with iron homeostasis.[27] Folate was primarily suggested for the counteraction of neural absconds.[28] Folate is an imperative donor of the methyl group and thus therefore it is vital for protein and DNA union. Another job by donating a

methyl group, transfer an extra carbon atom to homocysteine and transform it into methionine.[29] Diminished folate intake or hereditary variations in the gut for the folate digestion were related to the higher serum concentration of homocysteine.[30] While in preeclampsia the levels of homocysteine were seen enhanced.[31,33] There is little information on the relationship of folate to preeclampsia, but current trials are being done in the United States and different nations to find the lessened effect of pre-eclampsia with folate intake. In short, Nutrients were somehow associated with the risk factors of preeclampsia, but we cannot rely on small sample size research trial, but large-scale trials needed a huge funding which is certainly un-available for these research focuses.

The aim of our study is to see the effects of nutritional supplements such as LNS (Mamta) on angiogenic factors in pre-eclamptic women in our community to prevent negative outcomes by early diagnosis and treatment, because being the high incidence of pre-eclampsia and its negative outcome (i-e eclampsia, renal failure, liver failure, disseminated intravascular coagulation, abruptio placentae, intrauterine growth retardation, preterm delivery and stillbirth) in Pakistan till date no other study found.

MATERIAL AND METHODS

Single blinded randomized control trial (RCT) was designed to find the association of sFLT-1 to placental growth factor at Pre-eclamptic women (*n=84*) in KPK population with the addition of LNS at Interventional arm (*n=42*).

A total of eighty-four *(n=84)* pre-eclamptic participants with positive test of proteinuria (pre-selection) were recruited for our study RCT from Saidu Group of Teaching Hospital Swat, Zakir Khan Shaheed Hospital Matta & Civil Hospital Kabal swat. Further, women who were already diagnosed Pre-eclamptic and after 20 weeks of gestation were included in the randomized trial. However, essential hypertension and diabetes mellitus participants were excluded from our study. Schema of the trial is graphical presented in Fig 1.0. Height, weight were measure according to the standard operating protocols already discuss.[34] sFLT-1 &PlGF levels was measured with commercially available ELISA kit (Cat Log). Categorical variables were presented as count and percentage.

The comparison (t-test) was used to compare categorical and descriptive variables between the two groups. Al the test was done using Minitab ®version 17.

RESULTS

Table 1: Descriptive statistics of Anthropometry of the Study Participants (*n=84*) before& after Intervention

Study participants	Pre-eclamptic Control before Patients (*n=42*) Mean ± S. D	Pre-eclamptic interventional before patients (*n=42*) Mean±S.D	*p-value**	Pre-eclamptic Control after Patients (*n=42*) Mean± S. D	Pre-eclamptic interventional after patients (*n=42*) Mean±S.D	*p-value**
Weight (kg)	74 ± 9.0	67.2 ± 8.1	0.00	75.1 ± 9.0	69.1 ± 7.7	0.001
Height (ft)	154 ± 8.6	154 ± 5.7	1.000	154 ± 8.6	154 ± 5.7	1.000
BMI	31 ± 4.0	28 ± 3.5	0.00	31. 3 ±4.0	28.8 ± 3.3	0.00
Age at Marriage	23.3 ± 3.1	17.5 ± 2.9	0.00			
Age at Pregnancy	24.5 ± 3.8	24 ± 5.6	1.000			
sFlt-1 pg/ml	68.8 ± 215.9	57.9 ± 207	0.810	161 ± 322.9	184 ± 866	0.871
PLGF pg/ml	107.7 ± 63.0	97.97 ± 59.82	0.450	104.7 ± 57.2	107 ± 113.4	0.877
sFlt-1/ PLGF	0.900 ± 2.88	0.48 ± 1.66	0.313	1.71 ± 3.15	0.57 ± 1.9	0.034
Systolic Bp	137.9 ± 7.6	163.7 ± 12.8	0.00	116.7 ± 4.7	141.0 ± 9.1	0.00
Diastolic Bp	95 ± 6.7	108.2 ± 9.9	0.00	75 ± 4.0	93.53 ± 8.0	0.00
Gestation Weeks	35 ± 2.9	26.1 ± 3.2	0.00	38.9 ± 2.8	30.1 ± 3.2	0.00
Hb g	10.3 ± 1.4	12.0 ± 1.7	0.00	11 ± 1.6	11.6 ± 1.9	1.000
Platelets	268395±204629	200140±61551	0.041	259140 ± 42833	298047±69359	0.003
WBC		11.3 ± 2.5	----		10.3 ± 2.0	----

Table 2: Tally of the discreet variables

Study participants	Pre-eclamptic Control before Patients (n=42) Mean ± S. D	Pre-eclamptic interventional before patients (n=42) Mean ± S.D
Female Education Primary Secondary Higher uneducated	 4 (9.30%) 8 (18.60%) 9 (20.93%) 22 (51.16%)	 12 (27.91%) 6 (13.95%) 3 (6.98%) 22 (51.16%)
Female Occupation Housewife Job	 33 (76.74%) 10 (23.26%)	 41 (95.35%) 2 (4.65%)

Diabetic Yes No	0 (0%) 43 (100%)	0 (0%) 43 (100%)
Hypertensive Yes No	16 (37.21%) 27 (62.79%)	0 (0%) 43 (100%)
Heart Diseases Yes No	0 (0%) 43 (100%)	0 (0%) 43 (100%)
Kidney Diseases Yes No	0 (0%) 43 (100%)	0 (0%) 43 (100%)
Swelling on Face Yes No	0 (0%) 43 (100%)	30 (69.77%) 13 (30.23%)
Swelling on Feet Yes No	0 (0%) 43 (100%)	43 (100%) 0 (0%)
Husband Education Primary Secondary Higher uneducated	1 (2.33%) 17 (39.53%) 14 (32.56%) 11 (25.58%)	6 (13.95%) 16 (37.21%) 10 (23.26%) 11 (25.58%)
Pay 0 5000 10000 20000	5 (11.63%) 22 (51.16%) 16 (37.21%)	 4 (7%) 22 (51.16%) 17 (38%)
Husband Occupation Businessman Farmer Driver School Teacher Shop Job	5 (11.63%) 3 (6.98%) 6 (12%) 24 (56%)	3 (6.98%) 5 (11.63%) 2 (4.65%) 6 (13.95%) 12 (27.91%) 15 (34.88%)
No of Prenatal Visits 2 3 4	17 (39.53%) 26 (60.47%)	20 (46.51%) 18 (41.86%) 5 (11.63%)
Expecting Twins Yes No	0 (0%) 43 (100%)	0 (0%) 43 (100%)
Blood in Urine Yes No	3 (6.98%) 40 (93.02%)	5 (11.63%) 38 (88.37%)
Dizziness Yes No	12 (27.91%) 31 (72.09%)	27 (62.79%) 16 (37.21%)
Abdominal Pain Yes No	35 (81.40%) 8 (18.60%)	14 (32.56%) 29 (67.44%)
Severe Headache Yes No	11 (25.58%) 32 (74.42%)	31 (72.09%) 12 (27.91%)
Reduced Output of Urine Yes No	12 (27.91%) 31 (27.91%)	4 (9.30%) 39 (90.70%)
Vomiting Yes No	41 (95.35%) 2 (4.65%)	22 (51.16%) 21 (48.84%)
Nausea Yes No	24 (55.81%) 18 (41.86%)	26 (60.47%) 17 (39.53%)
Seizures Yes No	15 (34.88%) 28 (65.12%)	0 (0%) 43 (100%)
Coma Yes No	12 (27.91%) 31 (72.09%)	0 (0%) 43 (100%)
Rapid wt. Gain Yes No	35 (81.40%) 8 (18.60%)	40 (93.02%) 3 (6.98%)
Visual Yes No	11 (25.58%) 32 (74.42%)	36 (83.72%) 7 (16.28%)
Cardiovascular Yes No	2 (4.65%) 41 (95.35%)	11 (25.58%) 32 (74.42%)
Hepatic Yes No	12 (27.91%) 31 (72.09%)	4 (9.30%) 39 (79.07%)
Cerebrovascular Yes No	35 (81.40%) 8 (18.60%)	4 (9.30%) 39 (79.07%)
Obstetrical Yes No	11 (25.58%) 32 (74.42%)	22 (51.16%) 21 (48.84%)
Hemostatic Yes No	2 (4.65%) 41 (95.35%)	9 (20.93%) 34 (79.07%)
FamHx Hypertension Yes No	18 (41.86%) 25 (58%)	25 (58.14%) 18 (41.86%)
FamHx of PIH Yes No	15 (34.88%) 28 (65.12%)	17 (39.53%) 26 (60.47%)

Table 3: Gestation Weeks

Study participants Variables	Pre-eclamptic Control Patients (*n=42*) Count (%)	Pre-eclamptic interventional patients (*n=42*) Count (%)
Gestation weeks 27- 30 31- 35 >36	 2 (4.6%) 18 (41%) 23 (53.4%)	 39 (90.70%) 4 (9.30%)

Table 4: Comparison of Pre-eclamptic Control patients before & after intervention

Study participants	Before Intervention	After Intervention	*p-value*
	Pre-eclamptic Control Patients (*n=42*) Mean± S. D	**Pre-eclamptic Control Patients (*n=42*) Mean± S. D**	
Weight	74 ± 9.0	75.1 ± 9.0	0.608
Height	154 ± 8.6	154 ± 8.6	1.000
BMI	31 ± 4.0	31. 3 ±4.0	1.000
sFlt-1 pg/ml	68.8 ± 215.9	161.5 ± 322.9	0.266
PLGFpg/ml	107.7 ± 63	104.7 ± 57.2	0.817
sFlt-1/PLGF	0.900 ± 2.88	1.7 ± 3.15	0.213
Systolic Bp	137.9 ± 7.6	116.7 ± 4.7	0.00

Diastolic Bp	95 ± 6.7	75 ± 4.0	0.00
Hb	10.3 ± 1.4	11 ± 1.6	0.00
Platelets	268395 ± 204629	259140 ± 42833	0.773

Table 5: Comparison of Pre-eclamptic interventional patients before & after intervention

Study participants	Before Intervention	After Intervention	
	Pre-eclamptic interventional patients (*n=42*) Mean± S.D	**Pre-eclamptic interventional patients (*n=42*) Mean± S. D**	***p-value***
Weight (kg)	67.2 ± 8.1	69.1 ± 7.7	0.221
Height (cm)	154 ± 5.7	154 ± 5.7	1.000
BMI	28 ± 3.5	28.8 ± 3.3	1.000
sFlt-1 pg/ml	57.9 ± 207	184 ± 866	0.349
PLGF	97.97 ± 59.82	107 ± 113.4	0.609
sFlt-1/PLGF	0.48 ± 1.66	0.57 ± 1.9	0.792
Systolic Bp	163.7 ± 12.8	141.0 ± 9.1	0.00
Diastolic Bp	108.2 ± 9.9	93.53 ± 8.0	0.00
Hb	12.0 ± 1.7	11.6 ± 1.9	0.00
Urinary Protein 1+(30-100 mg/dl) 2+(100-300 mg/dl) ± (15-30 mg/dl) -ve (0mg/dl)	29, (67%) 4, (9%) 10, (23%)	5, (11%) 4, (9%) 34, (79%)	
WBC	11.3 ± 2.5	10.3 ± 2.0	0.023
Platelets	200140±61551	298047±69359	0.00

DISCUSSION

We have observed serum concentrations of sFlt-1, PlGF and the sFlt-1/PlGF-ratio in control and interventional arms, altogether with PlGF essentially decreased at control and increased in the interventional PE women. As per past discoveries,[35,36] serum levels are fundamentally modified, in cases with serious PE as well as in cases with gentle PE, pregnancies. These outcomes are in accordance with past discoveries of Rana *et al*.[37] exhibiting a higher sFlt-1/PlGF proportion of pregnancies with PE (n=46). In correlation with our outcomes, a sFlt-1/PlGF proportion was distinguished in control vs intervention [(1.71±3.15) vs (0.57±1.9) p=0.00)]. In the PE cases the investigation of Rana *et al*., mean levels of sFlt-1 increase in both arms, and PlGF, were higher only at interventional arm, while the sFlt-1/PlGF proportion did not vary at the baseline.

A conceivable pathophysiological clarification for the higher sFlt-1 and PlGF levels may be the increased placental mass that happens in pregnancies, also considered the root cause to pre-eclampsia (placental imbalance).[38] An immediate relationship between placental weight and concentration of angiogenic factors is as yet a point of controversy[39] (we have limitation in our investigation that we didn't gauge child birth weight).

Moreover, participants blood hemoglobin volume is fundamentally at the normal range during whole month trial in both the arms [control (Hb 10 versus 11 Hgmm)] [intervention (Hb 12 versus 11.6 Hgmm)]. Both PlGF and sFlt-1 is expanded. Notwithstanding, ELISA PlGF test just estimates free PlGF however not PlGF that were sFlt-1 bounded. Hence, the dimensions of PlGF estimated don't increment in connection to those of sFlt-1, which were increased.

Comparison of sFlt-1/PlGF ratio in those between the two groups shows significant relation the results found are in consistent with. While studies such as Bdolah *et al*.[40] demonstrating a fundamentally more elevated amount of sFlt-1/PlGF ratio in the maternal serum which is not the case in our study, but no difference in the level of PlGF& sFlt-1 were found significant.

Pre-eclampsia is considered a highly risk disease during a high blood pressure as well proteinuria (> 30 mg/dl). To know how the lipid-based supplements like Mamta, can impact the outcome of proteinuria, as to reduce it because we hypothesized that LNS can make a lowering of the pre-eclamptic effects. Now based on our results in the cross-sectional trial pre-eclamptic patients were about 23% in the normal range (15-30 mg/dl), but a majority of the portion i.e. 77% were having abnormal protein at their urine.

Interestingly, when the PE patients were feed with one-month trial on the lipid-based nutrients the results were very significant as it reverts back the abnormality in proteinuria to 11 %, and also increases the percentage from before 23% to after 88%, so this was a very huge change in the protein category. This might suggest a possible way to overcome the symptoms which were associated with the pre-eclampsia, as the literature suggests protein restriction (proteinuria) in pre-eclamptic patients are the good way for this disease because protein helps in the growth and fluid balances in transport mechanism, also, patients were more prone to the disease symptoms having hypoalbuminemia that lowers the osmotic pressure (imbalance).[41] However, there are some controversies in the literature that did not significantly co-relate the malnutrition and pre-eclampsia,[42] however our study results are conflicting with that.

This examination is one of its kind of investigations that measures the sFlt-1 and PlGF levels at swat in pre-eclamptic women of Swat KPK with intervention on lipid-based supplements for one month. Our readings have important baseline information for the clinical practice of suspected PE pregnancies. Current cut-offs being used for the analysis of PE were set up with information from singleton pregnancies. When looking at the precision and specificity of the sFlt-1/PlGF ratio at distinguishing the PE women from both the groups at our examination lacks significant information at the particular area, although studies we found checked contrast difference.[43] The finding that mean PlGF levels were not changed when looking at intervention and control uneven result data adds critical information to the talk about the utilization of single markers versus marker mixes in the exact determination of PE. While a few analysts propose that assurance of PlGF alone is adequate to analyze PE precisely, others see a reasonable addition of an angiogenic and as well antiangiogenic factor.[44]

The information in our study utilize, markers blend to analyze PE precisely with or without intervention (LNS), While particularly as the utilization of PlGF alone would prompt false-positive outcomes in pregnant pre-eclamptic women.

Our investigation has certain limited factors. Like, attributable to the low number of cases, and time allotment for sample intervention i.e. one month. In like manner, PE pregnancies exhibited at altogether impact with differential at the gestational ages (33±3weeks versus 30±4weeks), which may hypothetically have some effect on examinations among these investigation type studies, although distinctions in the sFlt-1/PlGF concentrations estimated inside fourteen days of each other might be viewed as insignificant. Further stratified cluster randomized controlled trial, at over a large sample size were recommended to measure the expected affirm of our discoveries, which eventually provide a baseline data.

CONCLUSION

In conclusion, sFlt-1 and PlGF is as of now (latest) the most encouraging biomarkers for a better finding of hypertensive issue in pregnancy, especially PE. Cut-offs score with the changes in sFlt-1/PlGF ratio may clinically utilize these markers for PE pregnancies with or without intervention (LNS). sFlt-1/PlGF proportion is more critical among control and intervention with an unaltered mean PlGF level in control pre-eclamptic patients. We infer that the analytic window in the utilization of the sFlt-1/PlGF ratio differs with LNS supplementation.

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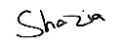
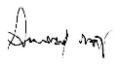
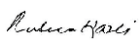
1	Shazia Hanif	Data collection, lab work and manuscript writing	
2	Amin ul Haq	Main idea, Data analysis & supervision of the manuscript writing	
3	Rubina Nazli	Data analysis, Critical review and final correction of the manuscript	
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