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The effect of homocysteine , some vitamins in women who suffer from recurrent miscarriage in Thi-Qar province / Iraq

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Abstract:

Recurrent miscarriage is the occurrence of two or more consecutive losses during the first or second trimester of pregnancy and affects 5% of couples and 1% have three or more consecutive miscarriages. This process is exhausting and both psychologically and physically disturbing for the couple, since 50% of recurrent miscarriages have unknown causes,But our research included increase homocysteine resulting from a deficiency of B vitamins (B_{12} and Folic acid) as hyperhomocysteinemia is associated with diseases that are mediated by the placenta, such as preeclampsia or placental abruption. Therefore, the aim of the study was to measure the levels of homocysteine in women who had two or more consecutive miscarriages and to evaluate the effect of vitamin B supplementation on homocysteine. To find out if lowering homocysteine can give good outcomes in women suffering from recurrent miscarriages of unknown causes.

study design

The study included (135) women (45 control and 90 patients). The women suffering from two consecutive miscarriages or more, were divided into two groups, 45 pregnant women and 45 non-pregnant women, which were compared with 45 pregnant women (control group) who did not suffer from a previous miscarriage and had at least one previous birth. Miscarriages for known causes were excluded from our study

Results

The results showed a significant increase in the level of homocysteine in the group RPL pregnant compared to the control group $P \leq 0.05$ and the level of vitamin B_{12} and folic acid significantly decreased compared to the control group. Also, there was a significant increase in homocysteine serum in the PRL non-pregnant group compared to the control group ($P \leq 0.05$) and a significant decrease in the level of vitamin B_{12} and Folic acid compared to the control group.

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In addition, the results showed an inverse relationship between the level of homocysteine and B vitamins (B_{12} - Folic acid).

Conclusion

Hyperhomocysteinemia is a risk factor for recurrent miscarriage (RM) with low B vitamins(B_{12}, folic acid). Vitamin B supplements may be beneficial for women with a history of recurrent pregnancy loss.

Keywords: Recurrent miscarriage - Homocysteine - Vitamin B_{12} – Folic acid.

Introduction:

Recurrent pregnancy loss (RPL), defined as two or more consecutive pregnancy losses [1]. Some experts consider two consecutive pregnancy losses is sufficient for the diagnosis of recurrent miscarriage (RM) because the recurrence rate and risk factors are similar to that after three losses [2].RPL is a distinct disorder that can be identified by two or more failed clinical pregnancies [3] Recurrent miscarriage can occur at any stage of pregnancy, it could be early or late pregnancy period. During the first trimester of pregnancy if there is loss of embryo then it is called as early pregnancy loss. If there is loss of fetus after first trimester of pregnancy it is called as late pregnancy loss. A frequency of late pregnancy losses is less as compare to early losses and consist of only 1% of pregnancies [4]. There exists a small number of accepted etiologies for RPL. Most of the diagnosed etiologies include endocrine abnormalities, autoimmune disorders, uterine anomalies, and genetic factors. After evaluation for these causes, approximately half of cases will still remain unexplained [5]

Maternal hyperhomocysteinemia (HHcy) is frequently associated with placental mediated diseases, such as recurrent miscarriage and preeclampsia [6] Vitamin B_{12} deficiency can affect pregnancy for both the fetus and the mother, since deficiency is a risk factor for intrauterine growth retardation or premature labor [7]. Low folate during pregnancy is associated with multiple risks of placental abruption and intrauterine growth restriction [8]

Homocysteine, a sulfur-containing amino acid derives from the demethylation of methionine during Deoxyribonucleic acid DNA or/and Ribonucleic acid RNA methylation [9], It is an amino acid not supplied by the diet that can be converted into cysteine or recycled into methionine , with the aid of specific vitamins B. When homocysteine levels are greater than normal limits, it signifies that there is a disruption in the metabolism of homocysteine[10]. There are several forms of homocystineHcy present in human blood. The free sulfhydryl form, homocystineHcy, is present in low amounts in blood (1%–2%) as a protein-bound mainly bound to albumin (about 70% to 85% in normal subjects) and soluble disulphides (30% -25%; e.g., homocystiene or cysteine). The amount of total homocystineis the sum of these three components [11].

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Elevated plasma total homocysteine can arise from inadequate folate or vitamin B_{12} status, but also from a range of other nutritional, genetic, physiological, and pathological causes. There is substantial circumstantial evidence to suggest that high circulating totalhomocysteine may itself increase the risk of a wide range of abnormalities in vascular function, most likely through increased oxidative stress leading to endothelial cell dysfunction. In pregnancy this is seen primarily as affecting placental function [12]. These levels are linked to the effect of homocysiene on vascular endothelial function, elevated pro-oxidant and thromboembolic activities [13]

Vitamin B_{12} (cobalamin) is a water-soluble vitamin obtained through the ingestion of fish, meat, and dairy products, as well as fortified cereals and supplements It is coabsorbed with intrinsic factor, a product of the stomach's parietal cells, Vitamin B_{12} is crucial for neurologic function, red blood cell production, and Deoxyribonucleic acid DNA synthesis, and is a cofactor for three major reactions: the conversion of methylmalonic acid to succinyl coenzyme A; the conversion of homocysteine to methionine; and the conversion of 5-methyltetrahydrofolate to tetrahydrofolate[14]Cellular deficiency in vitamin B_{12} is caused by inadequate intake, malabsorption, chemical inactivation, or inherited disruption of either B_{12} transport in the blood or intracellular metabolism[15]

Recent studies have also found an association between low vitamin B_{12} status in mothers and neural tube defect. This suggests an increased risk for birth defects when starting pregnancy with a deficient or inadequate vitamin B_{12} status [16].A vitamin B_{12} deficiency results in elevated concentrations of homocystein and methylmalonic acid in serum [17].

Folic acid (FA) is an oxidised synthetic form of the vitamin, which does not exist in nature, being only found in fortified foods, supplements and pharmaceuticals. Folic acid (FA) and dietary folate lack the ability to act as a substrate until they have been absorbed from the gastrointestinal tract and hepatically converted to the metabolically active 5-methyltetrahydrofolate (5-methylTHF) [18]. Folic acid is crucial for proper brain functioning and plays an important role in mental and emotional health. It helps in the production of DNA and RNA, the body's genetic material, especially when cells and tissues are growing rapidly, such as during infancy, adolescence, and pregnancy. Folic acid works closely with vitamin B_{12} in making red blood cells and helps iron function properly in the body. Folic acid works with vitamins B_6 and B_{12} and other nutrients in controlling the blood levels of the amino acid homocysteine[19].

Due to the association of low plasma folate levels with a higher risk of miscarriage , the increased intake of folate supplements could make improvements in pregnancy outcomes [20].

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Patients and methods

This study was conducted atBint Al Hoda Maternity and Children Hospital and specialist clinics in Thi-Qar governorate in the south of Iraq and Biochemistry Laboratory at the College of Science / ThiQar University , IIn formed oral consent was taken for patients.

The study included 135 women aged between (18 - 40) years, and they were divided into three groups:

The first group : consists of 45 women who have had two consecutive miscarriages or more who are currently pregnant, the period between (8-20) weeks.

The second group: consists of 45 women who have had two or more consecutive miscarriages, who are not pregnant.

The third group: consists of 45 women who have not experienced a previous miscarriage and are currently pregnant at the same age as the first group and have at least one successful delivery, and are considered as a control group.

Women who had suffered recurrent miscarriages for known reasons were excluded. A questionnaire was taken for the patients that included (age - number of previous miscarriage - total number of children - months of previous miscarriage - other reasons for miscarrge).

Blood Sample collection The blood was collected from a 5 mL vein and placed in a tube gel, then the serum was separated by centrifugation (10 min at 4000 rpm) and the serum was divided into four fractions which were kept in a clean eppendrof tubes and stored at -20 ° C in a deep freezer for later use. For required measurements:

• Determination of homocysteine in serum Hcy: using ELISA technology by spectrophotometer

•Determination of vitamin B_{12} using cobas e411 technique electroluminescence (ECL)

•Determination of folic acid using cobas e411 by electroluminescence (ECL)

The statistical analysis The statistical analysis was performed by using the software of Statistical Package for the Social Sciences (SPSS). Version 23.The results were expressed as mean ± standard deviations (mean ± SD). With LSD test .The T test was used to compare parameters in different studied groups. Pearson's correlation (r) was applied to determine the relationship among the present study parameters. P-values ($P \leq 0.05$) were considered statistically significant.

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Results and Discussion

Homocystiene:

Table (1) and Figure (1) shows significant increase in the concentration of serum Hcy in RPL pregnant group in comparison with RPL non pregnant and control groups ($p \leq 0.05$). Also it was found a significant increase in the concentration of serum Hcy in RPL nonpregnant group in comparison with control group ($p \leq 0.05$). the result of this study agree with previous studies [21, 22]. While, in contrast to our results[23]

A plausible causes of recurrent pregnancy loss is high levels of serum homocysteine. High levels of homocysteine may be particularly related to nutritional influences , Malnutrition and malabsorption of folic acid or vitamin B_{12} or due to genetic factors such as mutation in the gene of methylene tetrahydrofolatereductase (MTHFR) enzyme ,The MTHFR gene mutation reduces enzyme activity and increases the concentration of homocysteine in the blood [24]. It is proven that hyperhomocysteinemia causes a three times higher risk of early pregnancy loss [25]. The risk associated with hyperhomocysteinemia may be due to fetal toxicity or due to the elevated ability of homocysteine to activate factor V and inactivate protein C, heparan sulfate, or due to cellular methylation that alters gene expression or due to poor incorporation of uracil into the DNA leading to damage. DNA[26]

Table (1): Serum homocystiene levels of control and patients groups

Groups	No.	Hcy (p mol/ml) Mean ±SD
RPL non pregnant	45	284.24±48.79
RPL pregnant	45	535.27±76.18
Control	45	229.40±42.94
LSD		20.23

No: Number of subjects.
SD : Standard deviation.
LSD:Least Significant Difference
RPL: Recurrent pregnancy loss

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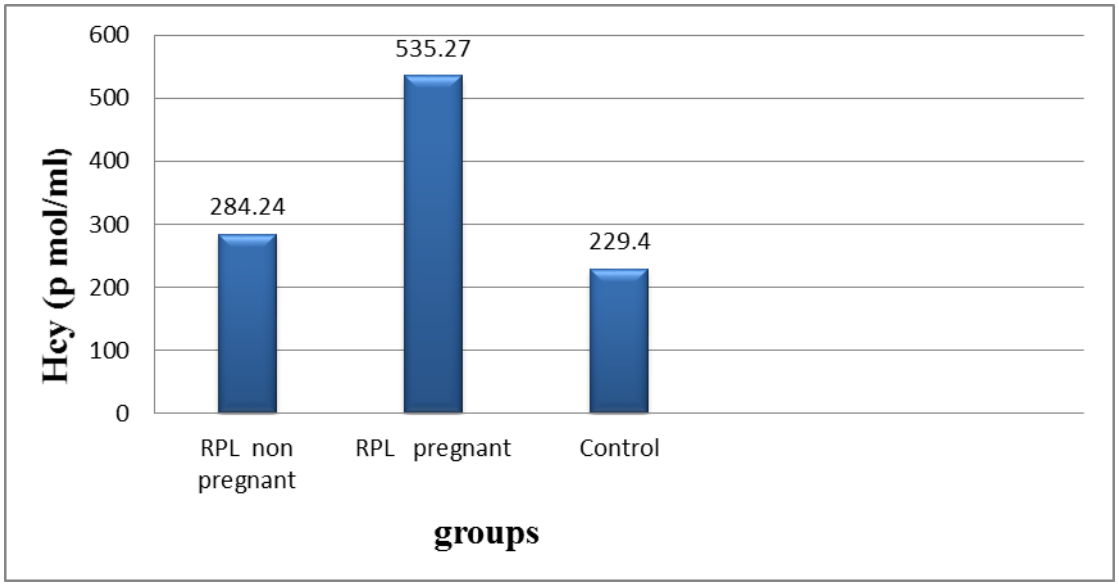

Figure 1: Serum Homocystiene levels of control and patient groups

Vitamin B_{12}

Table (2) and Figure (2)show significant decrease in the concentration of serum B_{12} in RPL pregnant group in comparison with RPL non pregnant and control groups ($p \leq 0.05$). Also it was found a significant decrease in the concentration of serum B_{12} in RPL non pregnant group in comparison with control group ($p \leq 0.05$).The result was consistent with previous studies[24, 25],Our results contradict a previous study [27].

Vitamin B_{12} participates in methionine metabolism and its deficiency is related to hyperhomocysteinemia, and it has been shown that vitamin B_{12} plays a role in recurrent pregnancy loss, as vitamin B_{12} deficiency leads to faulty and sporadic ovulation producing a faulty oocyte and vitamin B_{12} deficiency leads to incomplete trophoblastic invasion of spiral arteries thereby leading to defective placentation [25].

Table (2) Vitamin B_{12} levels of control and patients groups

Groups	No.	B_{12} (pg/ml) Mean ±SD
RPL non pregnant	45	260.49±46.66
RPL pregnant	45	149.01±30.91
Control	45	340.05±59.52
LSD		16.51

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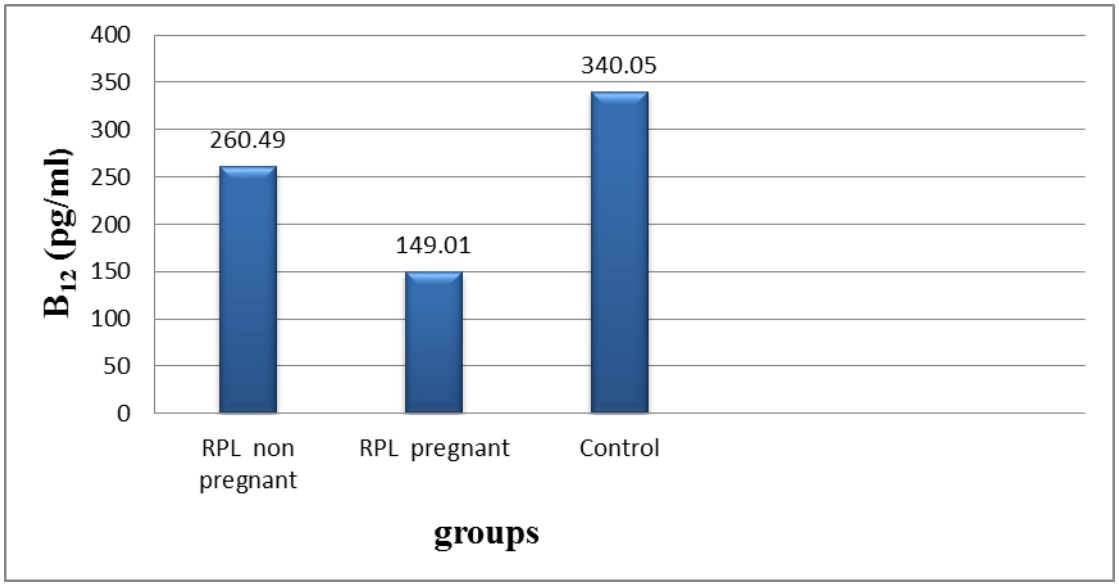

Figure 2 : Serum Vitamin B_{12} levels of control and patient groups

Table (3) and figures (3,4) shows the negative correlation between homocystiene and Vitamin B12 in RPL non-pregnant with correlation coefficient (r= -0.18 ,P.value=0.244) and RPL pregnant with correlation coefficient (r= -0.08 , P.value= 0.619).and this result indicates the possibility of using vitamin supplements to prevent further miscarriage in women with high homocysteine levels, and this result is consistent with [28]

Table(3) Correlation coefficient of Homocysteine and Vitamin B_{12}

Hcy with B_{12}	r	p. value	Results
RPL non pregnant	- 0.18	0.244	negative correlation
RPL pregnant	- 0.08	0.619	negative correlation

r: Pearson correlation coefficient

P. value : Probability

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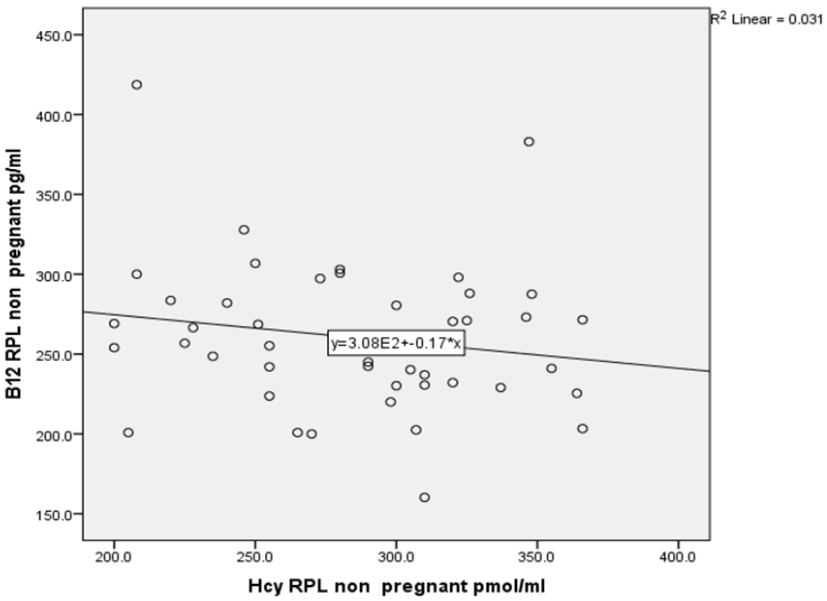
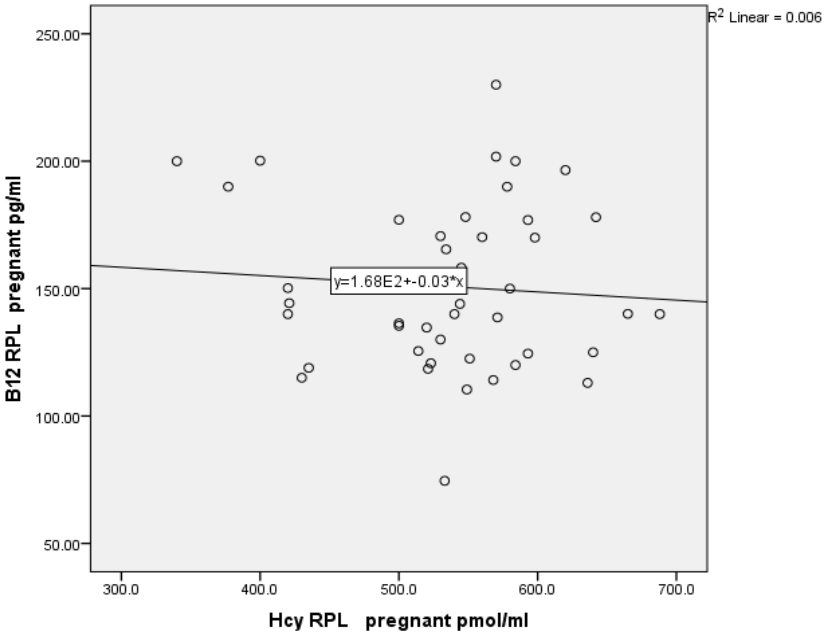


Figure(3): Correlation between serum Hcy and Vitamin B_{12} in RPL non pregnant group



Figure(4): Correlation between serum Hcy and Vitamin B_{12} in RPL pregnant group

Folic acid

Table (4) and Figure (5) shows significant decrease in the concentration of serum FA inRPL pregnant group in comparison with RPL nonpregnant and control groups ($p \leq 0.05$). Also it was found a significant decrease in the concentration of serum FA in RPL nonpregnant group in comparison with control group ($p \leq 0.05$).and this result is consistent with [22].

Our results contradict this studies[27, 28]

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Miscarriages are associated with a low level of Folic acid, which can have a toxic effect on the fetus in women suffering from recurrent miscarriage [29]. Observational studies have suggested that reduced circulating folate in pregnancy is associated with increased risk for preterm birth, placental abruption, intrauterine growth restriction (IUGR) and pre-eclampsia[8].

Folic acid (FA) is important not only for Deoxyribonucleic acid DNA, Ribonucleic acid RNA and protein synthesis, but also it is substantial in energy production and normal cell division. Therefore, it plays an essential role in ensuring the quality of oocyte, its maturation, embryo implantation and subsequent normal pregnancy [30].

Table (4) Folic acid levels of control and patients groups

Groups	No.	FA (ng/ml) Mean ±SD
RPL non pregnant	45	11.23±1.99
RPL pregnant	45	10.56±1.83
Control	45	12.16±1.91
LSD		0.65

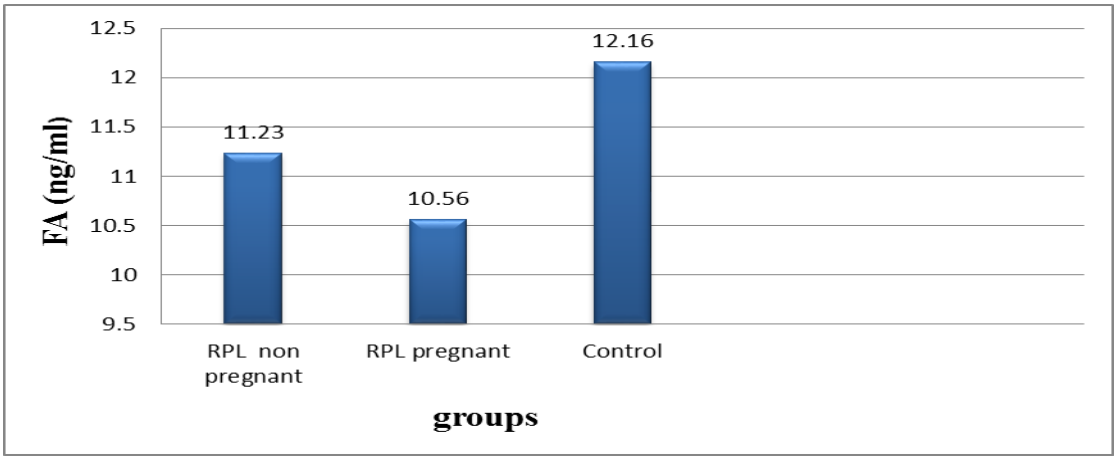

Figure 5 : Serum Folic acid levels of control and patient groups

Table (5) and figures (6,7) shows the negative correlation between homocystiene and folic acid in RPLnon- pregnant with correlation coefficient (r=-0.05 ,P.value=0.746) and RPL pregnant with correlation coefficient (r=-0.10 , P.value= 0.535).

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Table (5) Correlation coefficient of Homocysteine and Folic acid

Hcy with FA	R	p. value	Results
RPL non pregnant	- 0.05	0.746	negative correlation
RPL pregnant	- 0.10	0.535	negative correlation

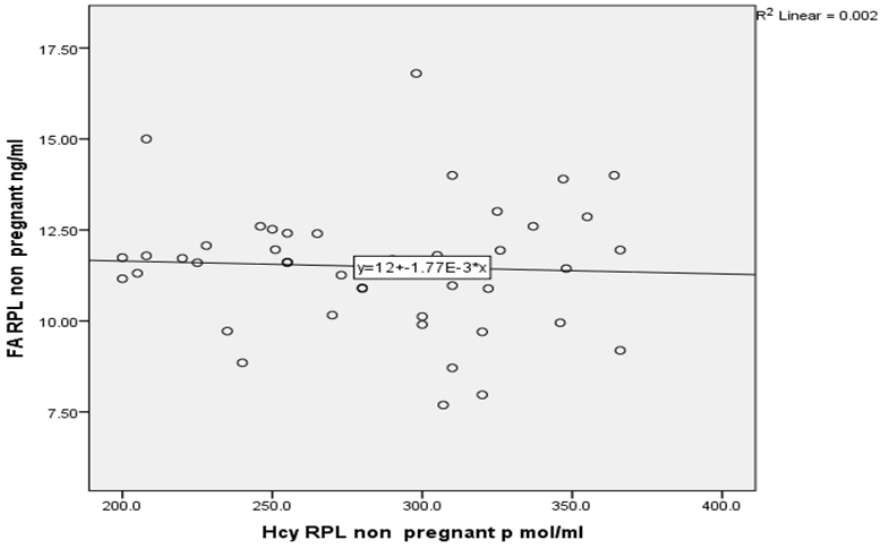

Figure (6): Correlation between serum Hcy and Folic acid in RPL non pregnant group

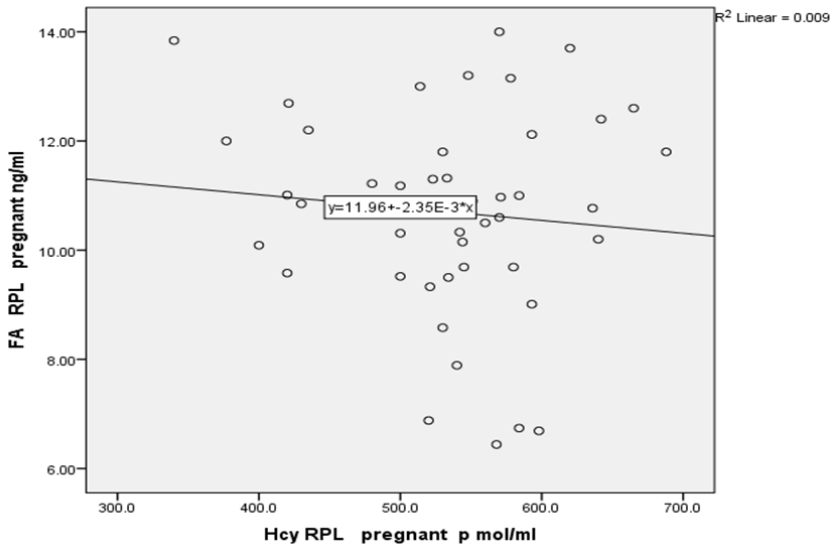

Figure (7): Correlation between serum Hcy and Folic acid in RPL non pregnant group

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