

# Expression of Micro RNAs 206 and 133b and serum IL-17 levels in preeclamptic females

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## ABSTRACT

Kidney diseases are common health problems around the world. Preeclampsia is defined by American college of obstetrics and gynecologists (ACOG) as a pregnancy specific hypertensive disease with multisystem disorder. It usually occurs after 20th week gestation most often near term and can be superimposed on another hypertensive disorder. Micro RNAs (miRs) are little, non-coding RNAs with 22 nucleotides in length with important functions in adjusting many biological processes. The gene for human miR- 206 is centralized on chromosome 6 in a bicistronic group jointly with the gene for miR-133b; gene IL-17 is downstream of 206/133b cluster. The aim of our study is to determine the change in the expression of miRNAs 206 and 133b in the placenta of preeclamptic females and in serum IL-17 and correlate them to the severity of preeclampsia. The present study was conducted on 80 pregnant females divided into 2 groups: Group I: which included 40 preeclamptic females and Group II controls which included 40 normotensive pregnant females. Both miRNA 206 and miRNA 133b are highly expressed in the placenta of preeclamptic Egyptian patients compared to the controls. Micro RNA 206 with 95% sensitivity and specificity, while Micro RNA 133b sensitivity was 90% and specificity was 95%, thus providing promising evidence that the expression of both parameters can be used as diagnostic markers for preeclampsia. The placental expression of miRNA 206 and miRNA does not seem to correlate to the severity of the condition. Similarly, serum level of interleukin 17 which was found to be increased in preeclampsia patients, and does not seem to correlate to the severity of the condition.

**Keywords:** Preeclampsia microRNA Interlukin17 Micro RNA206 Micro RNA133b.

## Introduction

Preeclampsia is defined by American college of obstetrics and gynecologists (ACOG) as a pregnancy specific hypertensive disease with multisystem disorder. It usually occurs after 20<sup>th</sup> week gestation most often near term and can be superimposed on another hypertensive disorder [1]. The disease is mainly due to defective placentation that leads to poor uterine and placental perfusion that contributes to the release of some antiangiogenic factors as a result of oxidative stress, which

leads to microangiopathy and endothelial dysfunction [2].

Micro RNAs (miRs) are little, non-coding RNAs, 22 nucleotides in length, with significant functions in adjusting considerable biological processes inclusive apoptosis, cell discrimination, and cell advancement. They perform their actions by binding to the 3' untranslated region either partially or completely promoting either degradation of mRNA transcripts or translational inhibition [3].

In preeclampsia many of these processes are impaired, so miRNAs can be used for better understanding the pathology of preeclampsia [4]. During early development of myocytes, the cluster of mi RNA 206/133b plays an important role in homeostatic maintenance of skeletal muscle [5]. The gene for human miR-206 is centralized on chromosome 6 in a bicistronic group jointly to the gene for miR- 133b [6]. IL-17 is a potent cytokine that is produced by T helper cells which increases in women suffering from preeclampsia [7]. The gene of IL-17 is downstream of 206/133b cluster [8]. The aim of our study is to determine the change in the expression of miRNAs 206 and 133b in the placenta of preeclamptic females and in

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serum IL-17 and correlate them to the severity of preeclampsia.

## Materials and Methods:

The present study was conducted on 80 pregnant females divided into 2 groups:

Group I: which included 40 preeclamptic females attending Kasr Al Ainy Hospital, Obstetrics and Gynecology Department and Group II controls which included 40 normotensive pregnant females.

In this study we tried to evaluate the usefulness of the differential expression of miRNAs 206 and 133b in the placentas of preeclamptic females and serum IL-17 and correlate them to the severity of preeclampsia. A blood sample was withdrawn from all subjects between the 32nd and 38th week of gestation.

Two milliliters of blood were delivered into centrifuge tubes, left to stand for ten minutes, centrifuged at 3500 rpm for 10 min at room temperature and stored at  $-80^{\circ}\text{C}$  until use.

Measurement of serum IL-17 by enzyme-linked immunosorbent assay (ELISA) Human IL-17 ELISA Kit was provided by Chongqing Biospes Co., Ltd, China, Catalog No.: BEK1123. miRNAs 206 and 133b expression and quantitation will be detected by real time PCR

### 1. miRNA extraction from samples:

- miRNA was isolated using miRNA extraction kit supplied by mirVana<sup>TM</sup> PARISTM Kit, ambion, USA.
- Sample preparation by adding 300 $\mu\text{L}$  of Binding Buffer solution (L3) to tissue sample, then mixed well by vortexing and 300 $\mu\text{L}$  70% ethanol was added.

## Assessment of quality and concentration of isolated RNA

Nanodrop<sup>®</sup> spectrophotometer was used to measure the absorbance of isolated RNA at 260 nm, 280nm and 230nm.

Detection and quantification of the amplified miRNAs 206 and 133b using real time PCR Reverse Transcription – Polymerase Chain Reaction (RT-PCR) Quantification using the TaqMan<sup>®</sup> MicroRNA Assays was done using two-step RT-PCR

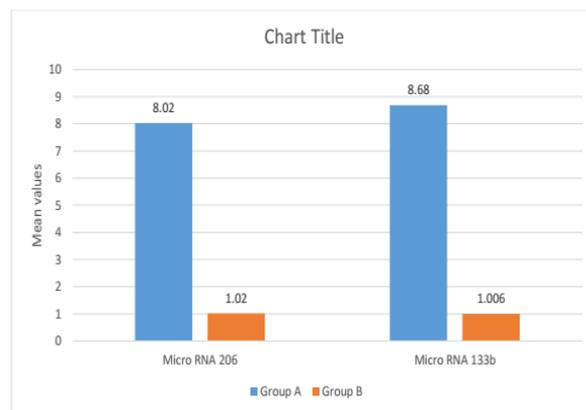
- In the reverse transcription (RT) step, cDNA was reverse transcribed from total RNA samples using specific miRNA primers from the TaqMan<sup>®</sup> MicroRNA Assays and reagents from the TaqMan<sup>®</sup> MicroRNA Reverse Transcription Kit.
- In the PCR step, PCR products were amplified from cDNA samples using the TaqMan<sup>®</sup> MicroRNA Assay together with the TaqMan<sup>®</sup> Universal PCR Master Mix.

## Results:

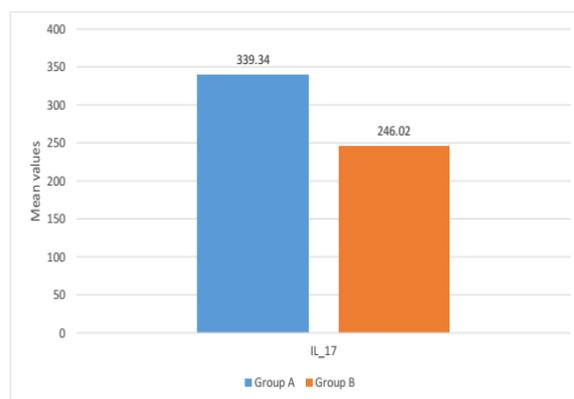
## Placental miRNA206, miRNA 133b and serum IL-17 are increased in patients with preeclampsia

The mean placental miRNA 206 and the mean placental miRNA 133b are significantly higher in preeclamptic females than in normotensive females with p value equals to (0.0001) Figure (1).

Also, the mean serum IL-17 is significantly higher in preeclamptic females than in the normotensive females with a p value equals to (0.002) Figure (2).



**Figure 1:** mean values of placental micro RNA 206 and micro RNA 133b in the studied groups.



**Figure 2.** Mean values of serum IL\_17 in both groups.

There was considerable development in the average standards of the systolic BP, diastolic BP and serum Albumin in the preeclamptic females compared to the control group while there was a significant decrease in the mean levels of the gestational age in the preeclamptic females compared to the control group, as regards albuminuria, serum haemoglobin, platlet count and INR there was no statistical difference between the two groups (table 1).

There is no statistical difference between the placental levels of miRNA206, miRNA133b and serum level of 133b in preeclampsia with severe features. Our results show that there is no significant difference between both placental miRNA 206 and miRNA 133b as well as serum IL-17 and severity of preeclamsiatale (2).

**Table 1: Comparison between the demographic, clinical and laboratory data of studied groups**

Dependent variables	Pre eclampsia Mean $\pm$ SD	Control Mean $\pm$ SD	P-value
GA (weeks)	35.65 $\pm$ 3.71	38.9 $\pm$ 0.91	0.001*
Systolic BP (mm Hg)	163.5 $\pm$ 17.85	112.75 $\pm$ 7.15	0.0001*
Diastolic BP (mm Hg)	105 $\pm$ 15.13	67.75 $\pm$ 8.34	0.0001*
Albumin Urine	2.15 $\pm$ 0.98	1.9 $\pm$ 1	0.488
HB (g/dl)	11.29 $\pm$ 1.78	10.92 $\pm$ 0.73	0.39
PLT( $\times 10^3$ /ul)	169 $\pm$ 71.27	184.45 $\pm$ 26.52	0.369
INR	1.02 $\pm$ 0.11	0.99 $\pm$ 0.01	0.319
Serum Albumin(g/dl)	2.81 $\pm$ 0.5	3.57 $\pm$ 0.07	0.0001*

**Table 2. The comparison between the serum IL-17, placental micro RNA 206 and placental Micro RNA 133b according to the clinical severity in the preeclamptic group**

	Mean Preeclampsia	Mean Preeclampsia with severe	p
IL17	367.5942	316.2394	0.222003
MicroRNA206	7.5656	8.4055	0.725194
MicroRNA133b	8.8122	8.5727	0.927741

An examination of the region down the curve worth founded on receiver operating advantage curves for each miRNA detected that placental miRNAs 206 yielded significant results, with AUC of 0.916 (p value < 0.0001) with sensitivity 95% and 95% specificity, in differentiating preeclampsia cases from healthy pregnant females which strongly suggest placental miRNAs 206 as efficient preeclampsia diagnostic biomarker.

An examination of the region down the curve worth founded on receiver operating advantage curves for each miRNA detected that placental miRNA133b yielded significant results, with AUC of 0.967 (p value < 0.0001) with sensitivity 90% and 95% specificity, in differentiating preeclampsia cases from healthy pregnant females which strongly suggest placental miRNAs 133b as efficient preeclampsia diagnostic biomarker.

Examination of the area under the curve (AUC) worth founded on receiver operating advantage curves for serum IL-17 detected that it is sensitive by 65% and specific by 75% and AUC was 0.774 with p value equals 0.002.

Using Chi square to detect the direction and Eta square to determine a relationship between severity and each of the placental micro RNAs 206 or 133b, there was no significant relationship between severity and placental microRNA 206 ( $X^2 = 0.134$  p=0.715) and placental micro RNA 133b ( $X^2 = 0.009$  p=0.925).

Using chi square and Eta square, there was only a significant relationship between systolic and diastolic blood pressure (p=0.012), and also albumin in urine (p=0.004) and gestational age (p=0.005) with the severity of the condition.

## Discussion:

As regards gestation age, McKinney *et al.* (2016) found that the gestation age were significantly decreased in their patients than in control group, which was matching with our results that show the mean gestational age in preeclampsia was (35.65 $\pm$ 3.71) while in control group was (38.9 $\pm$ 0.91) with p value =0.001<sup>[9]</sup>.

Our results showed that there was a statistically significant up-regulation in placental expression of microRNA 206 and microRNA 133b (p<0.0001) as well as statistically significant increase in serum IL-17 in patients with preeclampsia rather than the control group (p=0.002).

The finding that expression of miRNA 206 is significantly higher in preeclamptic females than normal pregnant females was in agreement with<sup>[10]</sup>, who showed that in preeclampsia miR-206 was greater in each of the circulations and the placenta and placental IGF-1 mRNA expression was lower-controlled in placental tissue. It is well determined that the pregnancy maternal IGF-1 has significant development influences on the fetus and myometrial vasculature, which are closely connected processes to the reasons of preeclampsia<sup>[11]</sup>. That research has experimentally supported reciprocal actions with a number of genes recognized to be immediately included in the pathology of preeclampsia, especially at the placenta: VEGF<sup>[13]</sup> NOTCH-3<sup>[14]</sup>, IGF-1<sup>[11]</sup> and hypoxia-inducible agent 1  $\alpha$  (HIF-1 $\alpha$ )<sup>[15]</sup>. This could be daring to be a physiological response to hypoxia in the placenta.

To the best of our knowledge there are no reports or studies on the efficacy of miRNA 133b in preeclampsia patients as diagnostic marker for diagnosis of preeclampsia.

A number of studies illustrated that aberrant expression level of miRNA in the placenta is associated with preeclampsia<sup>[16]</sup>. Some of the miRNAs are having been determined to be up and down-adjusted in preeclamptic placentas. Thumping expression of several miRNAs in placenta gives rise to the pathogenesis of PE with change key processes in placental advancement as lead to limited reproduction and insignificant infestation of trophoblast cells, additionally inadequate remodeling of maternal spiral arteries and defective angiogenesis<sup>[18]</sup>.

How miRNA expression is regulated in human placenta is not well understood. But researches documented that different reasons can control miRNA expression in placenta, including epigenetic regulation, oxygen tension, signaling molecules and environmental toxins. C19MC miRNAs expression pattern is associated with methylation status of a distal CpG-rich region located at 17.6 kb upstream of the miRNA cluster<sup>[19]</sup>.

Concerning to preeclampsia, the genomic shape in which miR-206 is established of attention. Moving in the direction in which a stream of miR-206 is the interleukin 17a (IL17a) gene that expresses IL-17, a potent cytokine mediating pro-inflammatory responses<sup>[20]</sup>. T- Helper cells that have been produced IL-17 (TH17) observed to be accessed in the circulation of females with preeclampsia. In addition to the same research, the cytokine itself was also found to be accessed in the circulation of a rodent model of preeclampsia<sup>[21]</sup>.

As regards serum IL-17 in our study, it has been increased (mean level in preeclampsia was  $339.34 \pm 91.73$  while in control group was  $246.02 \pm 88.34$ ).

The previous information on circulating IL-17 levels in preeclampsia was argumentative.

Our findings are harmonic with the previous researches confirming a greater prevalence of IL-17 levels in preeclampsias<sup>[22, 23]</sup>.

Excessed circulating IL-17 levels showed in preeclampsia could be contributing to the advancement of popularizing intravascular inflammatory responses advantage of the maternal syndrome of the illness. IL-17 induces pro-inflammatory reactions by triggering the expression of other cytokines (IL-6, granulocyte colony-activating agent, tumor necrosis factor- $\alpha$ ), chemokines (CXCL1, CXCL2, CCL20), inflammatory effectors (serious phase proteins, supplement) and antimicrobial proteins<sup>[22, 24]</sup>. Concerning, preeclampsia has previously been clearly showing the existence to be connected with great quantity of pro-inflammatory cytokines, chemokines, serious phase proteins, and supplement activation products in the maternal circulation<sup>[25]</sup>. Cornelius DC *et al.* (2014) reported that TH17 cells, likely via their secretion of IL-17 induce placental and renal oxidative stress which leads to production of agonistic autoantibodies to the angiotensin II type I receptor and placental vascular dysfunction resulting in the development of hypertension<sup>[26]</sup>. Molvarec A *et al.* (2015) previously observed that serum IL-17 levels were considerably greater in preeclamptic patients than in healthy non-pregnant and pregnant females<sup>[7]</sup>. They did not find any connection among serum IL-17 concentrations of preeclamptic patients and their clinical advantages and serum sFlt-1 and PlGF levels or sFlt-1/PlGF rates. Meanwhile, the greatest serum IL-17 level and sFlt-1/PlGF rate were reported to be additive for the danger of preeclampsia, as reported by the substantially increased strange rates of their incorporation than of either alone.

Most recently Darmochwal-Kolarz *et al.* (2017) reported that there were higher concentrations of IL-17 and lower concentrations of TGF- $\beta$  in patients with placental insufficiency when compared to control<sup>[23]</sup>. In the group of women patients with placental insufficiency, the levels of IL-17 positively relate with systolic blood pressure ( $r = 0.42$ ,  $p < 0.01$ ). Researchers concluded that it appears potential that the excesses concentrations of IL-17 and the shortage of TGF- $\beta$  in pregnancy complicated by FGR and PE can be answerable for the activation of inflammatory response showed in PE cases. Yang X *et al.* (2017) illustrated that the levels of miR-155 and IL-17 expression were established to be greater in preeclamptic placentas and serum, compared to the control group<sup>[27]</sup>. The results from this research explained a connection among miR-155 and IL-17 in proteinuria through slow onset of PE.

However, Ozkan *et al.* (2014) found that IL-35 and IL-17 levels of preeclamptic women were significantly lower compared to normal pregnant women<sup>[28]</sup>. They attributed this to the fact that the gestational age in their control group was

safety greater than that of the preeclamptic group. As circulating IL-17 levels have been reported to excess with improving gestation, the variation in gestational age might discompose their findings.

More research studies were done to measure IL-17 levels in serum of 13 preeclampsia women and 14 normal pregnant women with multiplex bead array, and no considerable variation was spotted<sup>[29]</sup>.

Talking about the relationship between serum IL-17 and severity of the disease our results found no statistical difference between preeclampsia and preeclampsia with severe features which was in concordance with Toldi *et al.* (2011) who found no statistically significant differences in the proportion of IL-17-producing lymphocytes between patients with preeclampsia and preeclampsia with severe features<sup>[22]</sup>. Also, Molvarec *et al.* (2015) could not find any relationship between serum IL-17 concentrations of preeclamptic patients<sup>[7]</sup>.

From the above results, it seems probable that miRNAs as mi206 and miRNA 133b could potentially aid the early detection of clinical conditions associated with pregnancy or fetal development. A substantial amount of miRNAs has been discovered in different body fluids in the form of cell free. These cell free miRNAs are stable and their expression profiles are various among different types of fluid, depending on tissue and biological stages. The other advantages of using miRNA biomarkers in non-invasive prenatal diagnosis is that simple detection and amplification methods, lower complexity, tissue-restricted expression profiles, and sequence conservation between humans and model organisms<sup>[30]</sup>.

## Conclusion:

Both miRNA 206 and miRNA 133b are highly expressed in the placentae of preeclamptic Egyptian patients compared to the controls. Micro RNA 206 with 95% sensitivity and specificity, while Micro RNA 133b sensitivity was 90% and specificity was 95%, thus providing promising evidence that the expression of both parameters can be used as diagnostic markers for preeclampsia. The placental expression of miRNA 206 and miRNA does not seem to correlate to the severity of the condition. Similarly, serum level of interleukin 17 which was found to be increased in preeclampsia patients, and does not seem to correlate to the severity of the condition.

## Perspectives:

Moreover, *in vitro* and *in vivo* studies utilizing particular miRNA weak cells are guaranteed to comprehensively comprehend the rates of the aberrantly expressed miRNAs in the beginning or advancement of preeclampsia. Whilst, it would be of considerable clinical benefit to over and above exam these little RNAs as possibility biomarkers for diagnosis of preeclampsia in a greater and prospective cohort.

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