

MicroRNA 210 expression profile from human placentas of pre-eclamptic pregnancies

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Abstract

Background: Pre-eclampsia (PE) is a common pregnancy specific syndrome that is characterized by hypertension and proteinuria. Evidence shows that pre-eclampsia is associated with alterations in placental microRNA expression. **Aims:** The purpose of this study was to compare the expression of the microRNA 210 in placentas from pre-eclamptic pregnancies versus normal placentas.

Patients and Methods: The study was carried out in the department of Gynecology and Obstetrics at Al-Yarmouk Teaching Hospital for the period from December 2009 to December 2010. Hundred pregnant women of age ranging from fifteen to forty years old, their gestational age ranging between twenty nine to forty weeks were divided into three groups: first group: mild Pre-eclampsia (twenty), second group: severe pre-eclampsia (thirty) & third group (fifty) normotensive pregnant women of comparable gestation were taken as a control. After delivery the Placentas from all patients (the study and the control groups) were obtained and studied for the microRNA 210 expression which was assessed by microRNA microarray and real-time reverse transcriptase-polymerase chain reaction analysis.

Results: MicroRNA 210 was over expressed in the placentas of pre-eclamptic pregnancies compared with normal placentas. In mild pre-eclampsia group the mean micro RNA-210 activity was 1.68 ± 0.25 , in severe pre-eclampsia group the mean microRNA-210 activity was 1.89 ± 0.13 and for the control group the mean microRNA-210 activity was 0.99 ± 0.15 . There was highly statistical significance in microRNA-210 activity of the studied groups since the P value for the mild pre-eclampsia versus the control was 0.0001. For the severe pre-eclampsia versus the control it was 0.0001 and for the severe pre-eclampsia versus mild pre-eclampsia was 0.0001.

Conclusions: The results showed that microRNA 210 was over expressed in pre-eclamptic pregnancies (mild and severe), which suggests its involvement in the pathogenesis of pre-eclampsia.

Keywords: MicroRNA, Placenta, Pre-eclampsia

INTRODUCTION

Pre-eclampsia (PE) is characterized by abnormal vascular response to placentation that is associated with increased systemic vascular resistance, enhanced platelet aggregation, activation of the coagulation system and endothelial cell dysfunction.^[1] Evidence shows that PE is associated with alterations in placental microRNA expression.^[2] MicroRNAs are noncoding RNAs of 21-24 nucleotides that function as negative regulators of gene expression by antisense complementarily to specific messenger RNAs.^[3, 4] The expression of microRNA-210 increase on exposure to hypoxia, which implies that microRNA-210 may be a potential marker of hypoxia. It is believed generally that poor placentation in pre-eclamptic pregnancies can cause focal regions of ischemia/hypoxia in the placenta. The increase of microRNA-210 expression may indicate the high degree of hypoxia in severe pre-eclamptic placentas.^[5]

Aim of the study

The study was conducted aiming to compare the microRNA-210 expression profile in placentas from women with mild, severe pre-eclampsia and normal pregnancy.

PATIENTS AND METHODS

This is a case control study conducted at the Department of Obstetrics & Gynecology at Al-Yarmouk Teaching Hospital for a period from (December 2009 to December 2010). The study included 100 pregnant women, who were admitted to the delivery suite. At the time of delivery, first group (twenty women) had mild PE, patient were considered as mild PE if their blood pressure value was equal or more than 140/90 mmHg in two readings six hours apart, and there was >+1 albumin in urine dipstick, second group (thirty women) had severe PE, patient were considered as severe PE if their blood pressure of equal or more than 160/110 with a proteinuria >+2 or in the presence of other concurrent parameters like persistent headache, visual disturbances, epigastric pain & impaired hepatic and renal function test,^[6] and third group (fifty normotensive pregnant women) their BP <140/90mmHg & proteinuria was nil, were taken as a control group, women in all the three groups had gestational age ranging between twenty nine to forty weeks. They were selected according to the clinical signs, symptoms & investigations. The inclusion criteria include: 1- Singleton pregnancy, 2- Maternal age from 15 to 40 years old & 3- Gestational age above

twenty weeks. The exclusion criteria include: 1- Anemia (hemoglobin <10.5 gm/dl), 2- Diabetes mellitus, 3- Multiple pregnancy & 4- Liver or renal diseases. The study was approved by the local Medical Research Ethics Committee of Al-Mustansiriya University, college of medicine, Department of Obstetrics & Gynecology. Informed consent was obtained from all participants before enrolling in the study. The demographic characteristic of each patient were assessed including maternal age, gravidity, parity, past medical, past surgical and past obstetric history. The gestational age was calculated on the date of last menstrual period or early ultrasonography. Complete examination was done and the following investigations were sent for the patients in all the three groups: Blood group and Rh, full blood count, liver function test, renal function test, and mid-stream urine for albumin. After delivery the whole placentas were taken, then put in container filled with formalin, then sent to the micro biologist who took snap-frozen placenta samples kept at -80°C. Small RNAs (200 nucleotides) were obtained with the mirvana RNA Isolation kit (Ambion, Austin, TX) according to the manufacturer's recommendations. Briefly, homogenized samples were lysed, followed by an acid-phenol: chloroform extraction. Total RNA samples were collected, and small RNAs were purified by precipitation with ethanol. Small RNA species were immobilized on glass-fiber filters, followed by several washes and elution of small RNA with nuclease-free water. In accordance with the mirvana protocol, Small RNA concentration was determined with the RNA 6000 Nano assay on the 2100 Bio analyzer. MicroRNA expression was determined with the Taqman microRNA Assays Human Panel–Early Access Kit (Applied Bio systems, Foster City, CA).

Statistical analysis

Data were analyzed using the computer facility of the available statistical software packages of SPSS-18 (Statistical Packages for Social Sciences-version 18) “PASW Statistics. Data were coded and entered in the computer, then presented in simple measures of frequencies and percentages for both patients and control groups. The values were expressed as mean, standard deviation minimum & maximum. The significance of difference in means was tested by the student t- test for two independent means while significance of difference between proportions was tested by the ANOVA test using $P \leq 0.05$ as the level of significance.

RESULTS

100 patients were included in the study. Table 1 shows the demographic characteristics of the three groups included in the study. The mean maternal age for mild PE was 30.20 ± 6.25 , for severe PE was 26.57 ± 6.64 and for the control group was 28.56 ± 5.91 . There was no statistically significant difference in the mean maternal age as the P value for mild PE Vs. control was 0.43, P value for severe PE Vs. control was 0.404 and P value for severe PE Vs. mild PE was 0.259. The mean parity for the mild pre-eclampsia was 1.80 ± 1.54 , for the severe pre-eclampsia group was 1.77 ± 1.52 and for the control group was 1.36 ± 1.40 . There was no statistically significant difference in the parity in between the groups since the P value for mild PE Vs. control was 0.640, for severe PE Vs. control was 0.585 and for severe Vs. mild PE was 0.999. The mean gestational age in the mild PE group was 36.30 ± 2.34 weeks, while for severe PE group was 35.13 ± 3.10 weeks and for control group was 35.60 ± 3.10 weeks. There was no significant difference in the gestational age of the studied groups since the P value for mild PE Vs. control was 0.124, for severe PE vs. control was 0.606, and for severe PE Vs. mild PE was 0.064.

Table 1. The demographic characteristics of the three groups in the study.

	Severe PE (n=30)	Mild PE (n=20)	Controls (n=50)	P- value
Age (years)	26.57 ± 6.78	30.20 ± 6.25	28.56 ± 5.91	>0.05
Mean $\pm$ SD	17-38	17-38	17-38	
(Range)				
Parity	1.77 ± 1.52	1.80 ± 1.54	1.36 ± 1.40	>0.05
Mean $\pm$ SD	0-4	0-4	0-4	
(Range)				
Gestational age	35.13 ± 3.10	36.30 ± 2.34	35.60 ± 3.10	>0.05
Mean $\pm$ SD	29-40	31-39	29-40	
(Range)				

*Significant difference between mean at 0.05 level of significance.

Table 2; figure 1 shows the relative microRNA-210 activity levels by RT-PCR for the three different groups. In the mild PE group the mean microRNA-210 activity was 1.68 ± 0.25 , while for severe PE group was 1.89 ± 0.13 and for the control group was 0.99 ± 0.15 . There was high statistically significant in microRNA-210 activity of the studied groups since P value for mild PE Vs. control was

0.0001, p value for severe PE Vs. control was 0.0001, and p value for severe PE Vs. mild PE was 0.0001.

Table 2. The relative microRNA-210 activity levels by RT-PCR for the three different groups.

	No.	MicroRNA-210		P-value compared	
		Mean $\pm$ SD	Range	To control	To mild PE
Severe PE	30	1.89 ± 0.13	1.61-2.11	0.0001*	0.0001*
Mild PE	20	1.68 ± 0.25	1.13-1.96	0.0001*	---
Controls	50	0.99 ± 0.15	0.63-1.50	---	---

*Significant difference between two dependent means using Student-t-test for two independent means at 0.05 level of significance.

DISCUSSION

Few investigators have studied placental microRNA expression in relation to pre-eclampsia and even fewer describe microarray-based global placental microRNA profiling in pre-eclampsia.^[7-9] The targets of microRNA210 are enriched in immune processes, which supports the association between abnormal immune responses and pre-eclampsia.^[10, 11] The current study showed significant difference in the expression of microR-210 in pre-eclampsic groups comparing to the control groups and the increment was more as the severity of pre-eclampsia increase. Our result was highly statistically significant since the P value was (0.0001). These findings are comparable to the findings of the Pineles et al. (2007)^[2] study which include 9 PE group, 9 small for gestational age (SGA) and 9 PE and SGA, the study showed that the expression of microRNA-210 increase on exposure to hypoxia at the same time, there was biochemical similarities between pre-eclampsia and SGA.^[2] Marked increase in the expression of microRNA-210 seen in isolated PE and PE associated with SGA indicate that these microRNA-210 may be related to the presence of pre-eclampsia, regardless of the presence of SGA. While Zhu et al. 2009^[12] study which include 8 patient with mild PE, 15 severe PE and 11 as a control group showed that the expression of microRNA-210 decrease in mild PE and

increase in severe PE. The study showed that the expression of microRNA-210 increase on exposure to hypoxia, the study shows that microRNA-210 may be a potential marker of hypoxia.

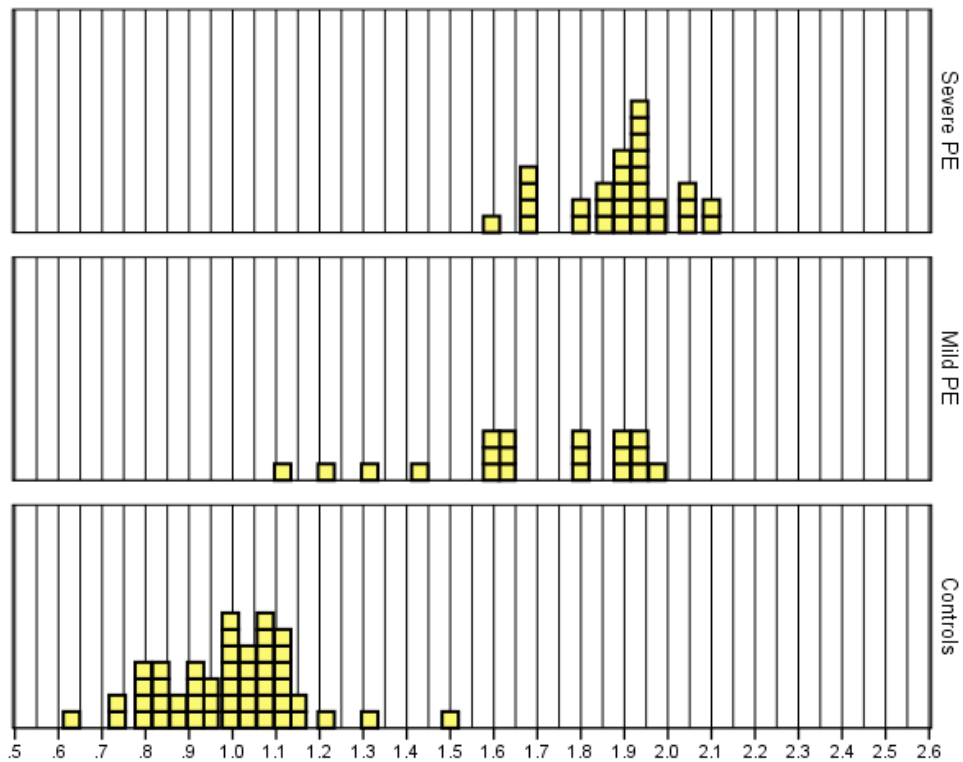


Figure 1. The relative microRNA-210 activity level distribution by RT-PCR for the three different groups.

It is believed generally that poor placentation in pre-eclamptic pregnancies can cause focal regions of ischemia /hypoxia in the placenta.^[12] The increase of microRNA-210 in severe PE may indicate the high degree of hypoxia in the placenta, whereas the decrease of microRNA-210 in mild PE placentas may be a compensatory mechanism in the pregnancies with mild PE. The results of the current study were in agreement with Hu et al at 2009^[13] study which investigate the placental microRNA-210 expression and risk of severe pre-eclampsia. They conclude that microRNA-210 expression is increased in severe PE.

In a study done by Daniel et al. at 2011^[14] which include 20 pre-eclamptic women and 20 normal women. His study showed evidence supporting previously reported that hypoxia is related to up-regulation of microRNA-210 roles in endothelial cell response to hypoxia, formation of capillary-like structures, vascular endothelial growth factor driven cell migration, cell

differentiation, and survival, events that are integral to pre-eclampsia pathogens expression. In a study done by Fei et al. at 2011^[15] showed that the expression of microRNA-210 is up-regulated in patients with pre-eclampsia, as well as in trophoblastic cells cultured under hypoxic conditions. According to his study, ectopic expression of microRNA-210 inhibit the migration and invasion capability of trophoblastic cells which related with cell migration and vascular remodeling, were then experimentally validated as the functional targets of microRNA-210 both *in vivo* and *in vitro*. The study showed an important role for microRNA-210 in the molecular mechanism of pre-eclampsia. Mayor-Lynn et al at. 2011^[16] study which include the expression profile of different microRNAs including microRNA-210 in placentas from patients with pre-eclampsia and preterm labour compared to normal term pregnancies. In his conclusion, the results provide further evidence that placentas affected by PE and preterm labor display an

altered expression of a number of microRNAs including microRNA-210 which show over expression in pre-eclamptic placentas with potential regulatory functions on the expression of specific target genes whose altered expression and function have been associated with these pregnancy complications. There was no overlap of identified microRNA-210 expression between their study and ours.

Conclusion

This study showed over expression of microRNA-210 in the placentas of severe and mild pre-eclamptic pregnancies Vs. placentas of normal pregnancies. The increased of microRNA-210 may indicate the high degree of hypoxia in severe pre-eclamptic placentas.

REFERENCES

1. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. *Am J Obstet Gynecol* 2000;183:S1-S22.
2. Pineles BL, Romero R, Montenegro D, Tarca AL, Han YM, Kim YM, et al. Distinct subsets of microRNAs are expressed differentially in the human placentas of patients with preeclampsia. *Am J Obstet Gynecol* 2007;196:261.e1-6.
3. Barad O, Meiri E, Avniel A, Aharonov R, Barzilai A, Bentwich I, et al. MicroRNA expression detected by oligonucleotide microarrays: system establishment and expression profiling in human tissues. *Genome Res* 2004;14:2486-94.
4. Lim LP, Lau NC, Garrett-Engle P, Grimson A, Schelter JM, Castle J, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 2005;433:769-73.
5. Fasanaro P, D'Alessandra Y, Di Stefano V, Melchionna R, Romani S, Pompilio G, et al. MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. *J Biol Chem* 2008;283:15878-83.
6. Robson C. S, Hypertension and renal disease in pregnancy, Edmonds D.K in Dewhurst's textbook of Obstetrics and Gynecology for postgraduates, 6th edition, Blackwell publishing, 1999; chapter15:166-185.
7. Giannakakis A, Sandaltzopoulos R, Greshock J, Liang S, Huang J, Hasegawa K, et al. microR-210 links hypoxia with cell cycle regulation and is deleted in human epithelial ovarian cancer. *Cancer Biol Ther* 2008;7:255-64.
8. Zhang C. MicroRNomics: a newly emerging approach for disease biology. *Physiol Genomics* 2008;33:139-47.
9. Zhang Y, Diao Z, Su L, Sun H, Li R, Cui H, et al. MicroRNA-155 contributes to preeclampsia by down-regulating CYR61. *Am J Obstet Gynecol* 2010;202:466.e1-7.
10. Ness RB, Sibai BM. Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia. *Am J Obstet Gynecol* 2006;195:40-9.
11. Kim YM, Romero R, Oh SY, Kim CJ, Kilburn BA, Armant DR, et al. Toll-like receptor 4: A potential link between "danger signals," the innate immune system, and pre-eclampsia?. *Am J Obstet Gynecol* 2005;193:921-7.
12. Zhu XM, Han T, Sargent IL, Yin GW, Yao YQ. Differential expression profile of microRNAs in human placentas from pre-eclamptic pregnancies vs. normal pregnancies. *Am J Obstet Gynecol* 2009;200:661.e1-7.
13. Hu Y, Li P, Hao S, Liu L, Zhao J, Hou Y. Differential expression of microRNAs in the placentae of Chinese patients with severe pre-eclampsia. *Clin Chem Lab Med* 2009;47:923-9.
14. Enquobahrie DA, Meller M, Rice K, Psaty BM, Siscovick DS, Williams MA. Differential placental gene expression in preeclampsia. *Am J Obstet Gynecol* 2008;199:566.e1-11.
15. Zhang Y, Fei M, Xue G, Zhou Q, Jia Y, Li L, et al. Elevated level of hypoxia-inducible microRNA-210 in pre-eclampsia: new insights into molecular mechanisms for the disease. *J cell mol med* 2012;16:249-59.
16. Mayor-Lynn K, Toloubeydokhti T, Cruz AC, Chegini N. Expression profile of microRNAs and mRNAs in human placentas from pregnancies complicated by preeclampsia and preterm labor. *Reprod Sci* 2011;18:46-56.