

## Adiponectin in maternal and fetal cord blood during pregnancy and its relation to fetal birth weight

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### Abstract

**Introduction:** Adiponectin has been implicated in the physiology of insulin resistance during pregnancy. Insulin resistance is developed in the last half of pregnancy. Insulin has a direct influence on fetal growth and proven in past studies. Hence we can state that the adiponectin also has a effect on fetal development and growth and since it is a key regulator in the insulin sensitivity. The aim of the present study is to increase the knowledge of role of adiponectin, especially in a physiologically demanding state of pregnancy, and study its effect on development of a fetus by virtue of its birth weight and gestational age during a normal pregnancy.

**Materials and Methods:** This open-ended non-comparative prospective study was conducted at the outpatient and inpatient department of Obstetrics and Gynecology as well as in the department of Biochemistry, Sawai Man Singh Medical College, Jaipur on 50 normal pregnant subjects and their neonates attending Obstetrics and Gynecology, department for routine checkups during the period of August 2014 to July 2015. Levels of adiponectin in maternal serum during mid-trimester and pre-labor stage of pregnancy were evaluated and compared. The evaluation of maternal and fetal adiponectin levels were also done in relation to birth weight and gender.

**Results:** Adiponectin is detectable in pregnant subjects, with significantly higher levels seen in newborns; however, there was no relationship seen between maternal and fetal adiponectin levels at any stage of gestation (Midterm  $p=0.604$ , Full-term  $p=0.589$ ). Mean Birth weight of newborns was noted at 2.95 ( $\pm 0.445$ ) kg with slightly lower values in male babies. Statistically significant positive correlation was seen between fetal birth weights and fetal adiponectin levels ( $p<0.01$ ).

**Conclusion:** Adiponectin is a plausible candidate for illuminating some physiologic and pathophysiologic conditions associated with pregnancy and fetal growth. Our study concludes with the hope of further breakthrough studies in the clinical application of this very interesting molecule in the management of various metabolic diseases of newborns and adults.

**Keywords:** Infant, Growth, Ponderal index, Insulin resistance.

### Introduction

Adiponectin (also denoted as Adipo Q, GBP-28, ACR p 30 and apM1) known as a protein which is encoded by *ADIPOQ* gene in humans.<sup>1</sup> It is stated in previous studies to be known in regulating glucose levels along with fatty acid breakdown. Adiponectin enhances the body's response towards insulin. Levels of this hormone were inversely correlated to the percentage body fat in adults.<sup>2</sup> In a meta-analysis study it was found that there was no confirm association seen in healthy adults.<sup>3</sup>

The normal level of adiponectin is 5-10  $\mu\text{g/mL}$ . Plasma concentration reveals a sexual dimorphism, with males having lower levels than females.<sup>4</sup> Levels of adiponectin are higher in nondiabetics compared to diabetics.<sup>5</sup> There were increasing evidence supports that adiponectin plays an important role in regulation of the insulin sensitivity. In studies, it was found that there were an inverse relationship between plasma adiponectin levels and Insulin resistance.<sup>6</sup> One of the hallmarks of human pregnancy is insulin resistance.<sup>7</sup> The conventional view is that this metabolic change is the result of placental hormones such as Human Placental Lactogen (HPL).<sup>8</sup> However, during recent years adiponectin has been implicated in the physiology of insulin resistance during pregnancy.<sup>9</sup> Insulin resistance is developed in the last half of pregnancy, along with an increase in maternal weight.<sup>10</sup>

Insulin has a direct influence on fetal growth and proven in past studies. Hence we can state that the adiponectin also has a effect on fetal development and growth and since it is a key regulator in the insulin sensitivity. In the previous researches it was found that there was strong correlation between IUGR and adiponectin levels.<sup>12,13</sup> Hence, adiponectin levels correlate and proven to be an important link between adult metabolic syndrome and fetal size.<sup>14</sup> Limited information is available regarding adiponectin and intrauterine growth. We made an effort to study Adiponectin's presence in Indian female population and its putative role in most important physiological event of their life i.e. pregnancy and childbirth.

### Materials and Methods

This open-ended, non-comparative prospective study was conducted at the department of Obstetrics and Gynecology, SMS medical college Jaipur at Janana Hospital and Department of Biochemistry SMS Medical College, Jaipur on 50 normal pregnant subjects attending Obstetrics and Gynecology, department for routine checkups during the period of August 2014 to July 2015.

Consent was taken by the subjects in written and appropriate ethical approval was appropriately sought before the study.

A random selection of subjects for the study was made on basis of detailed history and proper clinical examination including pelvic examination, during their first visit to the antenatal clinic/labor room. Females who were obese (BMI>25 Kg/m<sup>2</sup>), diabetic, hypertensive and with a history of complicated pregnancies were excluded from the present study.

The present study was constituted with two steps:

**Step 1:** This study was conducted to estimate the adiponectin levels in maternal serum during the gestational period and the pre labor stage. The maternal adiponectin levels and fetal adiponectin levels were measured and compared.

**Step 2:** This study was conducted to estimate adiponectin levels in fetal cord blood and to evaluate its relation to gender, birth weight and ponderal index. Ponderal index (PI) in newborns (PI = weight (g) x 100/(height, cm)<sup>3</sup>) has been used as an indicator of fetal growth status, especially to assess asymmetrical intrauterine growth retardation. The PI, a measure of soft-tissue development, has been used for decades in research on the human neonate. Infants with unusually low PIs are underweight for their length (skinny); those with unusually high PIs are overweight (plump).<sup>15</sup>

From the 50 neonates 5mL cord blood was collected at the time of labor and before the placental separation. Gestational age was estimated according to the date of Last Menstrual Period (LMP) and correlated with ultrasound examination during the first trimester or may be in early part of second trimester.

The collected samples were centrifuged at 5000 RPM for 5 minutes at 37°C and serum were stored in a secure freezer at -20°C, after proper labeling, the samples were packed for use to perform adiponectin level in our SMS medical college's biochemistry laboratory, a standardized lab. Adiponectin levels were measured using the Quantikine Acrp 30 Immunoassay kit (R&D Systems Inc, Minneapolis, USA).

### Statistical Analysis

The findings of the study are reported as means ± SD. The statistical analysis was performed using 'SigmaStat version 3.5' data analysis software developed by SyStat

Software Inc, USA. The results of this present study as qualitative analysis in chi square test were used to find any relationship between study variables. For the analysis of continuous variables unpaired t-test and Mann Whitney Sum Rank test was used and comparisons between two groups with parametric data distribution e.g. male vs. female were interpreted with following statistical principles: p value < 0.05 was considered to be statistically significant result. (b) The pair (s) of variables with positive correlation coefficients and p values below 0.050 tends to increase together. (c) For the pairs with negative correlation coefficients and p values below 0.050, one variable tends to decrease while the other increases. (d) For pairs with p values greater than 0.050, there is no significant relationship between the two variables.

### Results

We studied 50 normal pregnant subjects and measured adiponectin concentrations in all mothers at various stages of gestations along with fetal cord adiponectin levels while comparing with various maternal and fetal parameters.

Demographically, the maximum number of cases was Literate (86%), From Urban background (74%), Younger age group (62%), Belonged to middle socio-economic class (82%). Mean (±SD) maternal age, Body Mass Index (BMI) and a gestational week of delivery were 24.40±3.9, 21.8±1.2 and 36.7±1.6 respectively. 55% were primigravidae, 29% were second gravidae followed by 16% multi gravidae. No change in adiponectin was noted with type of parity.

35 (70%) neonates were born by normal vaginal delivery while 15(30%) were delivered by Lower Segment Caesarean Section (LSCS), mostly by clinical indication such as previous caesarean section, breech presentation, placenta previa and some by maternal choice. But no statistical significance (p=0.113) was noted between the two groups Adiponectin levels.

BMI showed a rise with advancing stages of gestation. (92% had BMI 22-25 compared to just 4% at full term). Most of the pregnant subjects were between age group of 18-24 years (62%). No effect on Adiponectin levels was demonstrated with varying maternal age distribution.

**Table 1: Relationship between Maternal BMI & Maternal Adiponectin measured during various stages of gestation**

	AT 20-24 weeks (n=50)	At 36-40 weeks (n=50)
BMI of mothers (Mean ± SD)	21.8 ± 1.3 kg/m <sup>2</sup>	23.9 ± 1.1 kg/m <sup>2</sup>
Maternal Adiponectin (mean ± SD)	14.560 ± 2.865 µg/mL	10.460 ± 2.243 µg/mL
r value	-0.476 (negative) BMI &	-0.441 (negative)
p value*	0.00047	0.00136

\*Significant negative (p<0.05) correlation found between maternal BMI and maternal adiponectin levels.

Maternal BMI was measured twice during their pregnancy, at mid-term between 20-24 weeks (21.8±1.3Kg/m<sup>2</sup>) and at delivery stage between 36-40 weeks (23.9±1.1Kg/m<sup>2</sup>). This was concomitantly compared with their own serum adiponectin levels measured at the same time i.e. at midterm (14.560±2.865µg/mL) and then at full term (10.460±2.243µg/mL). Pearson correlation analysis

reported a statistically significant but negative correlation between the maternal adiponectin level and BMI at both stages of gestation (p= 0.00047, r= -0.476 and p= 0.00136, r= -0.441 respectively), implying that Adiponectin levels were lower with higher Maternal BMI with progressive gestation, thus proving our hypothesis.

**Table 2: Showing relationship between maternal serum adiponectin levels at mid gestation period and fetal adiponectin levels**

	Maternal adiponectin at mid-term (n=50)	Fetal adiponectin at delivery (n=50)
Adiponectin levels in Mean $\pm$ SD	14.56 $\pm$ 2.865* $\mu$ g/mL	57.26 $\pm$ 17.651* $\mu$ g/mL

\* p value and correlation coefficient = 0.604 and r value -0.0752, No correlation noted (p>0.05)

**Table 3: Showing relationship between maternal and fetal adiponectin levels at full term**

	Maternal adiponectin at full term	Fetal adiponectin at delivery
Adiponectin levels in Mean $\pm$ SD*	10.46 $\pm$ 2.243 $\mu$ g/mL	57.26 $\pm$ 17.651 $\mu$ g/mL
Adiponectin levels( $\mu$ g/mL) in Median <sup>#</sup>	11.0 $\mu$ g/mL	55.5 $\mu$ g/mL

\* p value and correlation coefficient = 0.589 (p>0.05) and r value 0.0790, No correlation noted. <sup>#</sup>on application of Mann

Whitney rank sum test p<0.001, T=3775.00 statistically significant difference in medians

Adiponectin is detectable in pregnant subjects, with significantly higher levels seen in newborns, however no relationship was noted between maternal and foetal adiponectin levels at any stage of gestation.

**Table 4: Comparison between fetal cord levels of Adiponectin and Birth weight**

	Female N=26	Male N=24	Total cases N=50
Birth Weight in Mean $\pm$ SD	2.96 $\pm$ 0.349 kg	2.90 $\pm$ 0.39 kg	2.95 $\pm$ 0.445 kg
Adiponectin in Mean $\pm$ SD	56.231 $\pm$ 14.583 ( $\mu$ g/mL)	58.375 $\pm$ 20.378 ( $\mu$ g/mL)	57.260 $\pm$ 17.65
p value	<0.001*	<0.001 <sup>#</sup>	<0.001 <sup>&amp;</sup>
r value (Pearson coefficient)	0.656 (positive)	0.801 (positive)	0.742 (positive)

\*<sup>#</sup>&p < 0.05, Significant correlation

Adiponectin was detectable in the cord blood of all the 50 neonates in concentration ranging from 27-112 $\mu$ g/mL. The mean and median levels were 57.2 $\pm$ 17.6  $\mu$ g/mL and 55.5 $\mu$ g/mL. Pearson coefficient when applied confirmed a strong relationship fetal birth weight of any gender and fetal adiponectin levels with a positive correlation (r= 0.742, p<0.001).

When comparing 26 female neonates to 24 male neonates included in this study, no significant (p= 0.672) difference were found in cord serum level of adiponectin.

Ponderal index is an important marker in assessing fetal growth states and consequently forms an important part of this study in defining any relationship with adiponectin and its fetal growth. Mean ponderal index in all the babies was 2.527 $\pm$ 0.126  $\mu$ g/mL with mean adiponectin levels measured at 57.260 $\pm$ 17.651  $\mu$ g/mL. Adiponectin show a strong and statistically significant correlation with ponderal index (p= 0.000245, r= +0.497).

## Discussion

In the present study an attempt was made to study about Adiponectin, an adipose tissue derived plasma protein with remarkable role in metabolic processes and also has anti atherogenic properties.<sup>16</sup> Although a lot has been done about this novel adipocyte hormone internationally but this area is quiet untouched in Indian population.

All mothers were normo weight with BMI of below 25 Kg/m<sup>2</sup>. However, the BMI showed a rise with advancing stages of gestation (92% had BMI 22-25 compared to just

4% at full term) but significant fall was noted in maternal adiponectin levels near full term period probably related to increasing weight of mother with consequent negative feedback mechanism of adipocyte on adiponectin secretion responsible for this finding. Similar results were found in a study done by Hendler I et al.<sup>17</sup>

The present study also provided evidence that serum adiponectin is present in maternal sample was same as other studies.<sup>18</sup> Another important finding of the present study is the relatively high adiponectin levels in the cord blood, two to six times higher than those in adolescents and young adults.<sup>19-21</sup> However no relationship (p>0.05) was noted between maternal and foetal adiponectin levels at any stage of gestation. Zhang ZQ et al found a weak positive correlation (r=0.172, P=0.004) in adiponectin levels in maternal vs. fetal circulation.<sup>22</sup>

The source of adiponectin in human body is still unknown and matter of research. The findings of present study suggest that adiponectin is mainly secretes from fetal tissues and no such evidence reported from placental and maternal tissues. These findings supporting the evidence of no correlation between the maternal serum adiponectin levels and cord blood adiponectin levels. In addition to the above stated findings, the adiponectin levels were significantly higher in in cord blood comparison to the maternal blood. Hence, it cannot be postulated that the high level of adiponectin in cord blood is due to the transport of adiponectin from the maternal serum to cord blood through placenta. Likewise, for the hypothesis of high level of adiponectin in cord blood is due to production of

adiponectin from the placenta is also not true. Since the separation of the feto-placental unit is occurred during labor, there is fall in the levels of serum adiponectin is observed after birth because of the removal of placenta and since the circulating half- life of serum adipocytokine is only few hours levels are decrease after the birth.<sup>22</sup> However, a study conducted by Sivan E et al reported that adiponectin levels at birth and levels 4 days post- delivery, shows no decline in adiponectin levels.<sup>23</sup> Moreover, a study by Corbetta S et al did not find adiponectin mRNA expression in placental tissues.<sup>24</sup>

This study has found a positive correlation ( $p < 0.001$ ) in between the birth weight and cord blood adiponectin. Some other researches also reported the same findings.<sup>23,25</sup> This is an very peculiar finding in relation to previously proven negative correlation between adiponectin levels and obesity in adults. The positive correlation was found between birth weight and cord blood adiponectin, which is comparable with the previous findings that the adipose tissue of newborns is mainly composed of newly differentiated small adipocytes which lacks the factors that were responsible for the adiponectin production inhibition. Similarly, various other studies had demonstrated that secretion of adiponectin from omental, but not from subcutaneous adipocytes which were also negatively correlated with BMI.<sup>26</sup> Since the subcutaneous adipose tissues are found abundantly in newborns, it can be the reason for the findings of adiponectin in cord blood and the correlation with birth weight due to the different distribution and differentiation of adipose tissue among newborns in comparison to adults. During fetal growth, glucose is the main energy source of fetus and is supplied continuously from the maternal circulation.<sup>27</sup> Insulin plays a significant role in increasing the uptake of circulating glucose by fetal muscle and adipose tissue. In addition in a previous study by Yamauchi T et al adiponectin receptors were found abundantly in human placenta.<sup>28</sup> Taken together, and given the significance of glucose and insulin in intrauterine fetal growth, these findings imply that adiponectin may have an important role in fetal growth and development. Our study also proved this hypothesis by show a significant positive correlation between Adiponectin and ponderal index ( $p < 0.001$ ). Mantzoros C et al showed a positive correlation<sup>29</sup> but Jia Zheng et al showed that levels of Adiponectin were not correlated with ponderal index ( $p = 0.606$ ).<sup>30</sup>

Therefore, although little information is available regarding the role of adiponectin in fetal growth, a high concentration of adiponectin in the fetus may be crucial to enhance the growth promoting effect of insulin through its insulin-sensitizing action.<sup>31</sup> The idea may explain the positive correlation between cord plasma adiponectin level and weight at birth as observed in this study as well.

Some findings in previous researches shows that adiponectin were gender dependent among adults,<sup>32,33</sup> for this reason, in present study we compared adiponectin levels between female and male newborns. But we did not found any statistical difference in adiponectin levels ( $p = 0.672$ ) between among both genders. The difference between the

adiponectin levels in adults may be because of the sex hormones effect<sup>11</sup> or other physiological and biochemical mediators which may be inactive or absent in newborns. There was no significant association was reported between mode of delivery and adiponectin levels. Hence, we can say that fetal stress during the vaginal delivery is not associated with different values of adiponectin levels. It was also observed that adiponectin levels estimated from cord veinous samples after birth were reliable marker of adiponectin levels during the similar conditions in-utero.

### Limitations

We couldn't rule out possible effect of circadian cycles of adiponectin, as we couldn't have data about the time of sample collection. Various studies have shown different role of different adiponectin isoforms.<sup>34,35</sup> We were not measure these adiponectin isoforms since, our ELISA kit could not differentiate high-molecular-mass complexes and low-molecular-mass trimer forms of adiponectin during estimation. Third, like to other earlier researcher's work, we did not consider and calculate fat mass or fat distribution by which, the connection between adiponectin and visceral fat in infants can be identified.

### Conclusion

Even with these limitations, our results present a description on adiponectin in maternal serum and cord blood of newborns. Adiponectin Levels were high in cord blood in comparison to the adults, similarly the cord blood adiponectin levels and birth weight of newborn have been well correlated. Therefore adiponectin may have some additional role in physiologic change during pregnancy and in the growth of fetus. However, the exact function of adiponectin by which it affects the human pregnancy and fetal growth is yet to be explained. Our study concludes with taking into consideration the parameters studied in this study of normal pregnancy and normal fetal outcome with adiponectin, it would be of interest to investigate cord blood adiponectin levels in the presence of intrauterine developmental abnormalities.

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