

Variations in Serum TGF β -1 Levels With respect To Gestational Age, Diastolic Blood Pressure and Parity in Normal Pregnant Women of a Tertiary Care Centre in North India

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Abstract

Background: Pregnancy is a physiological state that encompasses changes in the level of various cytokines in the body. TGF β -1 is an important cytokine in pregnancy. Studies have shown that during pregnancy TGF- β 1 plays an important role in trophoblast cell invasion, maintenance of fetal-maternal immune tolerance, and uterine spiral artery remodeling. We want to see whether TGF- β 1 level varies with changes in gestational age, diastolic blood pressure and parity or not. **Material and Methods:** Thirty normal pregnant women in their third trimester were selected from antenatal clinic of J.N. Medical College and Hospital, Aligarh. The study was conducted between September 2013 to March 2014. TGF β -1 was estimated with the help of an elisareader. Data was analysed statistically with the help of Graphpad Prism 5.0 software. **Results:** 1. Mean TGF β -1 levels in normal pregnant women were found to be 34.52 ± 2.61 ng/ml (N = 30). 2. Mean TGF β -1 levels in group1 were found to be 36.89 ± 1.80 ng/ml and in group2 were found to be 32.71 ± 1.37 ng/ml. This decrease in TGF β -1 levels with increase in gestational age was statistically significant (P value < 0.0001). 3. Mean TGF β -1 level in the group with DBP = 60-79 mm Hg was 48.62 ± 1.95 ng/ml and in the group with DBP ≥ 80 mm Hg was 48.41 ± 1.20 ng/ml. This difference in normal pregnant women was insignificant. 4. Mean levels of serum TGF β -1 in nulliparous pregnant women was 34.18 ± 2.15 ng/ml (N=15); and that in multiparous pregnant women was 34.89 ± 3.04 ng/ml (N=15). This difference in mean TGF β -1 levels was also insignificant (P value > 0.05). **Conclusion:** We conclude that in normal pregnancy serum TGF β -1 levels decrease with increase in gestational age but does not vary with increase in diastolic blood pressure and changes in parity.

Keywords: TGF β -1, DBP.

Introduction

Pregnancy is a physiological state of a human female that encompasses multiple physiological changes inside a female's body. These changes may be in the form of changes in blood parameters, anthropometric measurements, size of the organs³ or in the level of various cytokines in the blood. Transforming growth factor beta 1 or TGF- β 1 is a polypeptide member of the transforming growth factor beta superfamily of cytokines. It is a secreted protein from platelets and many leukocytes that performs many cellular functions, including the control of cell growth, cell proliferation, cell differentiation and apoptosis.^[1] S.Venkatesha et al (2006) found that TGF β -1 induces Nitric Oxide Synthase-dependent vasodilation in both renal and mesenteric resistance vessels.^[2] Studies have shown that TGF- β 1 plays an important role in trophoblast cell invasion, maintenance of fetal-maternal

immune tolerance, and uterine spiral artery remodeling.^[3] During healthy pregnancy, TGF- β 1 has four main functions 1. It induces the production of regulatory T cells. 2. It maintains the immunosuppressive function of regulatory T cells. 3. It mediates the balance of M1/M2 macrophages. 4. It regulates the function of NK cells.³ In this study we want to see the serum levels of TGF- β 1 in normal pregnancy and, whether they vary with respect to gestational age, diastolic blood pressure and parity, or not. Previous studies on this topic are limited or does not combine all these parameters together. Benian and Madazil (2002) found that TGF beta1 is increased in pre-eclamptic patients as compared to normal pregnant females and also that TGF beta1 increases with increase in diastolic blood pressure in pre-eclamptics.^[4] We want to see that whether TGF beta1 increases with DBP in normal pregnant females as well? Mandeep Singh et al. (2013) found that TGF beta1 decreased with increase in gestational age. Although the decrease in TGF beta1 was statistically significant before third trimester, but this decrease was not statistically significant in third trimester.^[5] We want to check that whether this is true for third trimester or not. Studies correlating TGF beta1 with parity are limited. Therefore, we want to see if there are any significant changes in TGF beta1 level as parity changes. This study will also provide levels of TGF beta1 in normal pregnant females of north Indian location.

Material & Methods

Thirty women with normal pregnancy in their third trimester and apparently good health were selected. They were selected from Antenatal Clinic of Obstetrics and Gynecology Department, J.N. Medical College Hospital, Aligarh. Informed consent (in accordance with the Helinski Declaration of 1975, revised in 1983) was taken from the cases and controls for participation in the study with approval of institutional Ethical Committee, J.N. Medical College Hospital, Aligarh. The study was conducted between September 2013 and March 2014.

Exclusion Criteria

1. Maternal age less than 20 years and more than 30 years.
2. Patients of Gestational Hypertension.
3. Patients with history of smoking and alcohol intake.
4. Duration of pregnancy less than 30 weeks.
5. Patients taking antioxidants.

Clinical History and Examination

Detailed clinical history and examination was done and age, parity, duration of gestation, past history suggestive of hypertension, diabetes or any concomitant complication of pregnancy were recorded. The following parameters were recorded to assess the patients:-

Age: Chronological age was recorded in completed years.

Height: Height was taken to nearest 1cm with a scale with the subjects standing on plain surface.

Weight: Weight was recorded to nearest 0.5 kg with the help of a weighing scale, subjects standing in erect position without shoes and wearing only light indoor clothes.

Mid-arm circumference(MAC): MAC was measured with the help of a measuring tape in centimeters with the subject in sitting position, arms by her side. MAC was measured at the level of mid arm taking acromion process and olecranon as reference points.

Blood Pressure (BP): BP was recorded with the help of a sphygmomanometer to the nearest 2 mm Hg with the subject at rest and in sitting or in left lateral recumbent position with the patient's arm at the level of heart. SBP (1st phase Kortkoff's sound) and DBP (5th phase Kortkoff's sound) was measured to the nearest even number.

E.Biochemical Analysis

The blood samples were collected in EDTA vials for serum TGF beta1 estimation. All the samples were centrifuged in the lab and serum was separated for study. Following biochemical parameters were estimated.

1. Serum TGF β -1: It will be measured in nanograms/ml (ng/ml) of serum.

Sample Collection

Intracubital venous blood (5ml) was collected from the subjects in supine position. The blood was allowed to be kept at 2 to 8 degree Celsius in a refrigerator for one hour. After this serum was obtained by centrifugation. Fresh serum were used for analysis of TGF beta1 by Elisa Reader.

Serum TGF β -1 Estimation

Serum TGF beta1 estimation was done with the help of Human TGF beta1 ELISA kit as described in the user manual (Revised Mar 1, 2012) by RayBiotech, Inc.^[6]

Principle of TGF Beta1 Estimation and Product Description

The Human TGF-beta1 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human TGF-beta1 in serum, plasma, cell culture supernatants. This assay employs an antibody specific for human TGF-beta1 coated on a 96-well plate. Standards and samples were pipetted into the wells and TGF-beta1 present in a sample got bound to the wells by the immobilized antibody. The wells were washed and biotinylated antihuman TGF-beta1 antibody was added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted to the wells. The wells were again washed, a TMB substrate solution was added to the wells and color developed in proportion to the amount of TGF-beta1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color was measured at 450 nm by the Elisareader.

TGF β -1 Sample Activation Procedure

To activate latent TGF β -1 to the immunoreactive form, we followed the activation procedure outlined below.

We added 0.1 ml 2.5 N Acetic Acid to 0.1 ml serum and mixed the tube thoroughly. Then we incubate it for 10 minutes at room temperature. Then we neutralized the acidified sample by adding 0.1 ml 2.7 N NaOH/1 M HEPES and mixed the tube thoroughly. Then, we did the assay immediately.

Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed in the user manual.
2. Add 100 μ l standard or sample to each well. Incubate 2.5 hours at room temperature or over night at 40C.
3. Add 100 μ l prepared biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 μ l prepared Streptavidin solution. Incubate for 45 minutes at room temperature.
5. Add 100 μ l TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature.
6. Add 50 μ l Stop Solution to each well.
7. Read at 450 nm immediately.

Statistical Analysis of Data

Results were analysed using appropriate statistical tests with the help of GraphPad Prism-5.0 software:

1. Mean
2. Standard Deviation (S.D.)

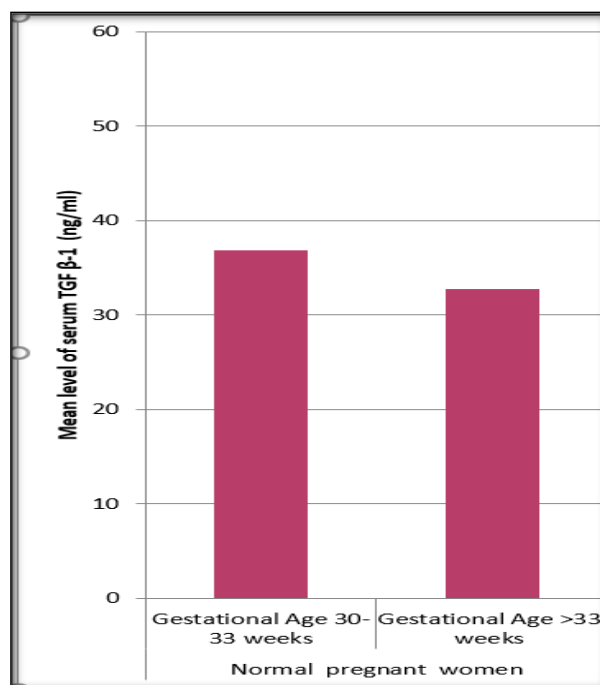
Unpaired t-test.

Results

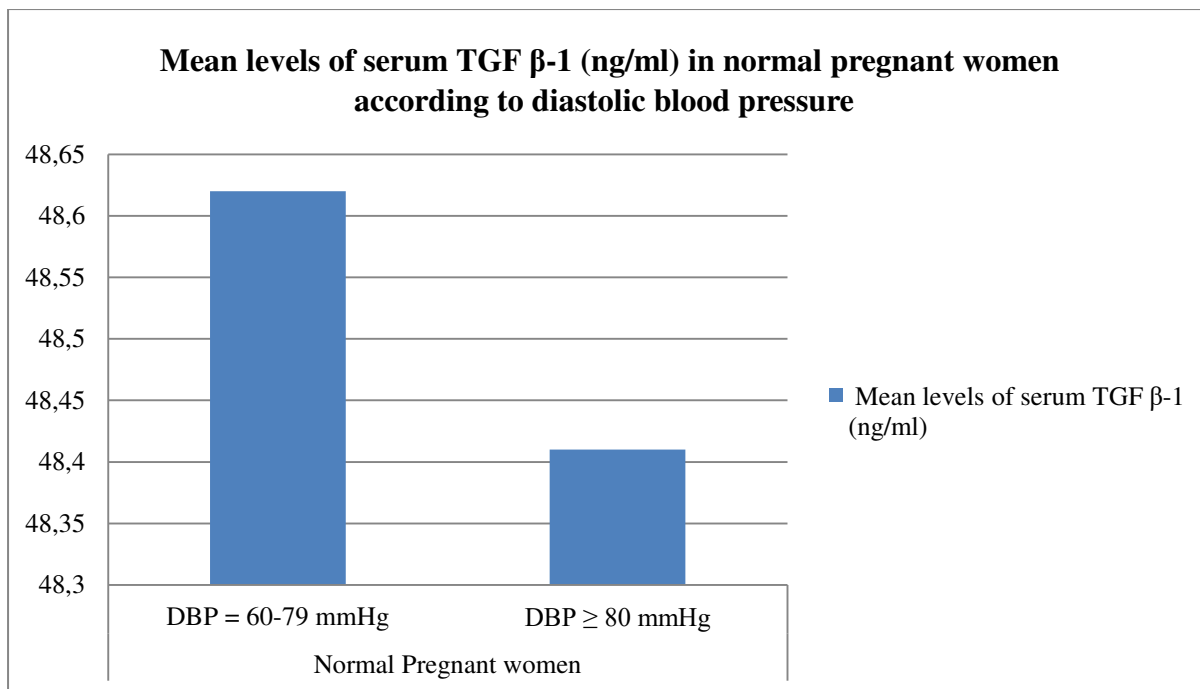
Table 1: General Characteristics of the subjects:

Characteristics	Mean value
Mean Age (years)	26 years
Mean Height (cm)	149 cms
Mean Weight (Kgs)	48.5 kgs
Mid-arm circumference (cm)	23.5 cms
Mean BP (mm Hg)	86 m Hg

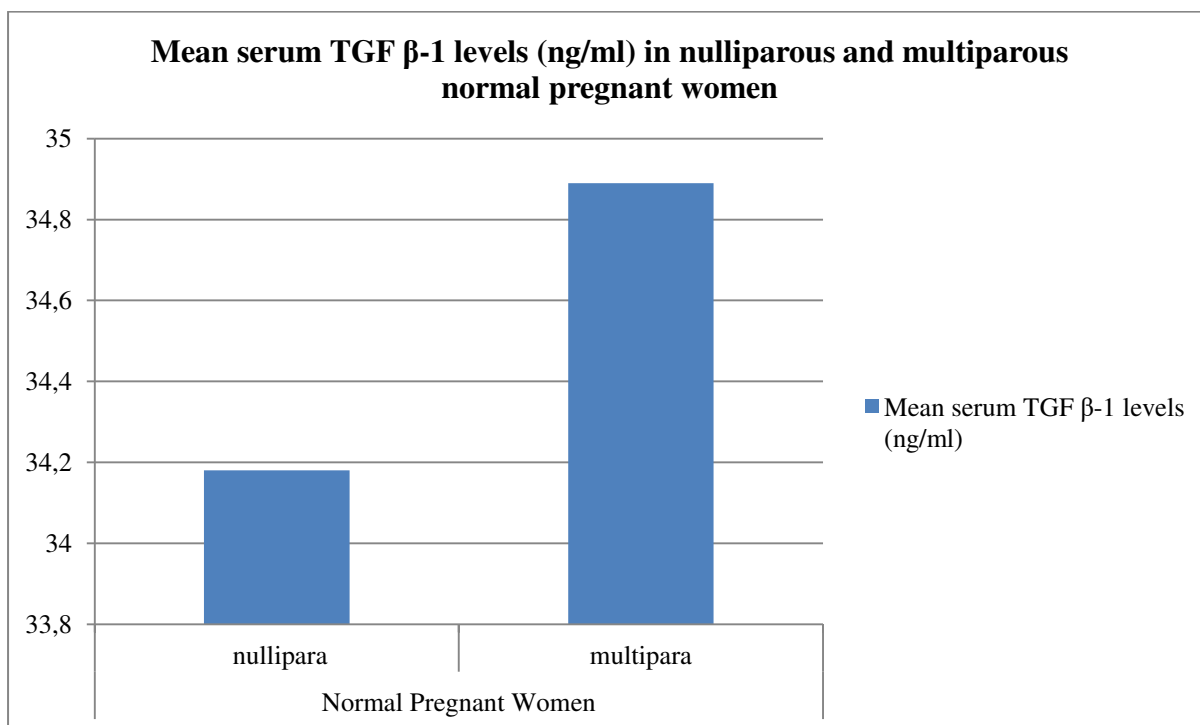
1. Mean TGF β -1 levels in normal pregnant women were found to be 34.52 ± 2.61 ng/ml (N = 30)
2. TGF β -1 levels of normal pregnant women were divided into two groups according to gestational age; Group1 with gestational age 30-33 weeks and Group2 with gestational age > 33 weeks. Mean TGF β -1 levels in Group1 were found to be 36.89 ± 1.80 ng/ml and in Group2 were found to be 32.71 ± 1.37 ng/ml. This decrease in TGF β -1 levels with increase in gestational age was statistically significant (P value < 0.0001).
3. Normal pregnant women were also divided into two groups according to diastolic pressure. Mean TGF β -1 level in the group with DBP = 60-79 mm Hg was 48.62 ± 1.95 ng/ml and in the group with DBP ≥ 80 mm Hg was 48.41 ± 1.20 ng/ml. This difference in normal pregnant women was insignificant.
4. Normal Pregnant women were also divided into nullipara and multipara and mean levels of TGF β -1 were calculated in both groups. Mean levels of serum TGF β -1 in nulliparous pregnant women was 34.18 ± 2.15 ng/ml (N=15); and that in multiparous pregnant women was 34.89 ± 3.04 ng/ml (N=15). This difference in mean TGF β -1 levels was also insignificant (P value > 0.05).



Graph 1: Mean levels of serum TGF beta1 in normal pregnant women divided on the basis of gestational age



Graph 2: Mean levels of serum TGF beta1 in normal pregnant women divided on the basis of diastolic blood pressure



Graph 3: Mean level of serum TGF beta1 levels in nulliparous and multiparous normal pregnant women

Discussion

We measured serum TGF β -1 levels in normal pregnant women. Our levels are consistent with previous studies.^[7] We divided normal pregnant women in two groups based on their gestational age. Women in Group1 had gestational age between 30-33 weeks and women in Group 2 had gestational age $>$ 33 weeks. We found that women in Group2 had lower levels

of serum TGF β -1 as compared to Group1. And this difference was statistically significant. This proves that serum TGF β -1 decreases with increase in gestational age. Our results are in line with previous studies of this pattern. Mandeep Singh (2013) found in their study that TGF β -1 levels fell significantly from 10-week to 26-week gestation, the change in the third trimester in the group as a whole was insignificant. They also found that serum TGF β -1 increased with gestational age in third trimester SGA pregnancies while it fell down with gestational age in AGA pregnancies. In our study all the pregnancies were Appropriate for Gestational Age (AGA). We also divided normal pregnant women into two groups on the basis of diastolic blood pressure (DBP). First group had DBP in the range of 60-79 mmHg and the second group had DBP \geq 80 mmHg. TGF β -1 was measured in both the groups but the difference in mean TGF β -1 levels in both the groups was not statistically significant. This proves that TGF β -1 does not increase with increase in DBP in normal pregnancy. We did not find any studies in support of this fact. Although there are many studies supporting the fact that TGF β -1 increase with increase in DBP in Pre-eclampsia but we did not find any study relating DBP and TGF β -1 in normal pregnancy. Further studies are required in this field. We also divided pregnant women on the basis of parity, say nullipara and multipara. We did not find any significant difference in mean serum TGF β -1 levels of nullipara and multipara groups. This proves that serum TGF β -1 does not vary with change of parity in normal pregnant women. We did not find any study in support of this fact. Further studies are required in this field. We did not include small for gestational age pregnancies in our study; with them the results would be different.

Conclusion

We conclude that in normal pregnancy serum TGF β -1 levels decrease with increase in gestational age but does not vary with increase in diastolic blood pressure and changes in parity.

The students who are unaware of the consequences of female feticide are at risk of continuing this practice as a legacy of their families. Hence there is ample scope of schools playing an active role in raising awareness among adolescents and is the need of the hour.

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