



Original Research Article

A study on intraocular pressure variation in primigravida and multigravida in trimesters of pregnancy and puerperium

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ABSTRACT

Objective: To study intraocular pressure variation in primigravida and multigravida in trimesters of pregnancy and puerperium.

Materials and Methods: This is a cross sectional and hospital based study of intraocular pressure (IOP) assessment of 693 female patients of non-pregnant, 1st trimester, 2nd trimester and 3rd trimesters of pregnancy and puerperium during routine ocular examination. This study carried out in nine groups of 77 in each group.

Results: In primigravida the mean IOP was 14.31 ± 1.78 mmHg, 14.82 ± 2.09 mmHg, 12.86 ± 1.75 mmHg in first, second and third trimesters respectively. In multigravida the mean IOP was 14.38 ± 1.97 mmHg, 14.36 ± 2.01 mmHg, 12.71 ± 1.63 mmHg in first, second and third trimesters respectively. In primigravida puerperal period the mean IOP was 14.55 ± 1.88 mmHg and in multigravida puerperal period the mean IOP was 14.04 ± 2.52 mmHg. Mean IOP during whole period of pregnancy was 14.00 ± 1.87 mmHg and 13.81 ± 1.08 mmHg in primigravida and multigravida respectively. Control group IOP was 15.92 ± 2.11 mmHg.

Conclusion: There is a trend of decrease of intraocular pressure during pregnancy and comes towards no pregnant level during puerperal period both in primigravida and multigravida. IOP decreased more towards second and third trimester of pregnancy. There was variation of IOP in primigravida and multigravida during pregnancy and puerperium. IOP was significantly low in multigravida than primigravida. The higher IOP in primigravida could be due to antenatal and postnatal anxiety and stress and sleeplessness. Pregnancy related effect on IOP is usual phenomenon and temporary.

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1. Introduction

Pregnancy is required for perpetuating human society; it is a period in which woman carries a developing fetus in uterus from conception to delivery. During course of pregnancy all the organic systems undergo complex physiologic changes which could be of innocuous and pathological.¹ It could affect cardiovascular, hematologic, metabolic, renal,

gastrointestinal, respiratory and endocrinal.² Changes more marked towards 3rd months of pregnancy due to release of hormones from placenta, maternal endocrine glands and fetal endocrine glands. The physiological or pathological process of changes also affect to ocular tissues. It could affect eyelid, conjunctiva, cornea, lens, optic nerve and tract resulting to changes in corneal sensitivity, refractive status, intraocular pressure, visual acuity etc. The ocular changes are very mild and transient not requiring any treatment. Some require treatment in pathological conditions. There

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is a tendency of decrease of IOP during pregnancy usually towards 2nd and 3rd trimesters and comes to base line by 2-3 months after delivery.^{2,3} This physiological change of decrease of IOP is a protective factor against Glaucoma.

Intraocular pressure plays important role to maintain normal physiological function of the eye maintaining vision. Aqueous is a clear fluid remains in anterior chamber (0.25ml) and posterior chamber (0.06ml). The normal aqueous derived from plasma of capillary network ciliary processes of rate of secretion 2.3 microlitre / minute. It flows from posterior chamber to anterior chamber through pupil. Drainage occurs through Trabecular (conventional) network and uveoscleral (unconventional) route. The normal IOP is maintained by dynamic equilibrium of formation and outflow of aqueous humor. The normal IOP varies between 10 to 21 mmHg.

It is observed that intraocular pressure change occurs during course of pregnancy which marked in 3rd trimester; probably due to increase uveoscleral outflow, decrease aqueous production. Hormonal effect plays a major factor. Ocular changes decrease corneal sensitivity and refractive error.^{1,4-6} Various studies showed decrease IOP during pregnancy which is highly marked in 3rd trimester.[1,2,8] Decrease of IOP could be due to increase of aqueous humor outflow, ligament relaxation/loosening, decrease of corneal rigidity, decrease of episcleral venous pressure and generalized acidosis.⁷ Vasodilatation due to pregnancy related hormonal effect hormones^{1,6,8-10} and pregnancy related metabolic acidosis.⁷ IOP comes to normal after delivery.

The aim of the study is to determine (a) whether systemic effect of pregnancy would affect IOP and to confirm ocular hypotensive effect of pregnancy. (b) Any IOP variation in different trimesters and puerperium both in primigravida and multigravida combined. (c) Any difference in primigravida and multigravida in different trimesters and in whole pregnancy. (d) Any difference in primigravida in all semesters with multigravida in all semesters in whole pregnancy period.

2. Materials and Methods

It is a cross-sectional and Hospital based study of patients from the ophthalmology and obstetrics and gynaecology outpatient department of district headquarter hospital, Jajpur district, and Odisha state. Written informed consent was taken from the patients. The study period was from 4th November 2022 to 30th April 2023. Study was approved by the Institutional Ethics Committee. Six hundred ninety-three patients were selected for the study. All female patients age ranging from 20 to 38 years with and without history of pregnancy were included in this study. Complete obstetric and antenatal history and history of delivery of primigravida and multigravida were taken. Females having preexisting glaucoma, family history of glaucoma,

old ocular trauma, high myopia and presence of systemic diseases like hypertension, diabetes mellitus and twin pregnancies etc. were excluded. Patients with preeclampsia, pregnancy induced hypertension and gestational diabetes were also not included in the study and managed.

There were six hundred ninety-three participants in the study groups consisting of 9 groups (Table 1): Each group consists of equal number of participants of 77(11.1%) numbers. Group1 first trimester primigravida; Group 2 first trimester multigravida; Group 3 second trimester primigravida; Group 4 second trimester multigravida; Group 5 third trimester primigravida; Group 6 third trimester multigravida; Group 7 puerperium primigravida ; Group 8 puerperium multigravida and Group 9 Control. 1st trimester (1st 14 weeks of pregnancy), 2nd trimester (14 to 28 weeks of pregnancy), 3rd trimester (> 28 weeks of pregnancy) and puerperium as postpartum period from end of delivery to 3-6 weeks period i.e., up to complete involution of uterus were defined. Patients were assessed in clinical and ultrasonography examination during antenatal period by Obstetrician. Patient's age and parity were recorded.

Complete ophthalmological examination was conducted to exclude any pathology in anterior and posterior segment. IOP was measured by non-contact tonometer; as it is a safer and valid diagnostic tool for IOP measurement.^{3,11} Different studies are showing IOP measurement by Schoitz tonometer^{10,12,13} and Applanation tonometer.^{1,3-5,14} Three readings of each eye were measured and average was recorded. Right eye was measured first. All examinations were performed in the morning hour of 8 to 10 AM.

Statistical package for social sciences (SPSS) software version 26 and M. S. Excel were used for data analysis. Numerical and categorical values were compared; p values were calculated to find the statistical significance of the tests. Un-paired t-tests were applied to find the statistical significance of intraocular pressure for different categories of subjects. A p value <0.0001 was considered significant.

3. Result

Demographic profile of study participants: Age- minimum 20, maximum 38 years. Overall patients were 693(100%). There were 231(33.3%) primigravida in three groups (Group-1, 3, 5), 231 (33.3%) multigravida in three groups (Group-2,4,6) in three trimesters, 154(22.2%) puerperium in two groups (Group-7, 8) and 77(11.1%) in Group 9 as control. Mean age of each group, study groups, overall age group, control group, in different trimesters and puerperium were calculated. The overall mean age was 29.05±4.00 years and study group 29.03±3.94 years having no statistical difference.(Table 1)

Describes the range and mean IOP of right and left eye of all the groups of the study participants. The range of mean IOP was minimum 8 mmHg and maximum 20 mmHg.

Mean IOP of overall participants N-693 (Group 1 to 9) was 14.19 ± 2.19 mmHg and study groups during pregnancy period both primigravida and multigravida combined (Group 1 to 6) 13.95 ± 2.09 mmHg, puerperium (Group 7,8) 14.21 ± 2.33 mmHg and in control (Group 9) 15.92 ± 2.11 mmHg. There was statistically significant difference of IOP in study group than control $p < 0.0001$. (Table 2)

Summarizes the result of IOP in different trimesters of both primigravida and multigravida combined. IOP in 1st semester both in primigravida and multigravida (Group 1,2) was 14.34 ± 1.87 , in 2nd semester both in primigravida and multigravida (Group 3,4) was 14.58 ± 2.06 , 3rd semester both in primigravida and multigravida (Group 5,6) was 12.79 ± 1.69 , puerperium (Group 7,8) was 14.21 ± 2.33 and in control (Group 9) was 15.92 ± 2.11 . The Intraocular pressure decreased over the period of pregnancy both in primigravida and multigravida being lowest in 3rd trimester. In puerperal period IOP raised from 3rd trimester significantly. (Table 3) It is statistically significant p value < 0.0001 . Statistically significant difference of IOP were observed within control group with group 1, 2 (both primigravida and multigravida), group 3, 4 (both primigravida and multigravida), group 5, 6 (both primigravida and multigravida) and with puerperium of both primigravida and multigravida with $p < 0.0001$ at 95% confidence limit

Compares the IOP of primigravida, multigravida in different trimesters and puerperium. (Table 4)

In 1st trimester mean IOP of both the eyes of primigravida (Group 1) was 14.31 ± 1.78 mmHg and in multigravida (Group 2) was 14.38 ± 1.97 mmHg. In 2nd trimester mean IOP of both the eyes of primigravida (Group 3) was 14.82 ± 2.09 mmHg and in multigravida (Group 4) was 14.36 ± 2.01 mmHg. In 3rd trimester mean IOP of both the eyes of primigravida (Group 5) was 12.86 ± 1.75 mmHg and in multigravida (Group 6) was 12.71 ± 1.63 mmHg. In 2nd and 3rd trimester IOP is lower in multigravida than primigravida is statistically significant with p value < 0.0001 . In puerperium period the mean IOP of both the eyes in primigravida participants (Group 7) was 14.55 ± 1.88 and multigravida participants (Group 8) was 14.04 ± 2.52 . The lower IOP in puerperium period of multigravida participants than primigravida is statistically significant p value < 0.0001 . Intraocular pressure comparison with control group were made from group 1, group 2, group 3, group 4, group 5, group 6, group 7 and group 8 both for primigravida, multigravida and puerperium of primigravida and multigravida. Significant low IOP were observed in primigravida and multigravidas in comparison to control group with $p < 0.0001$.

Control group IOP (Group 9) was 15.92 ± 2.11 . The mean IOP of study group (Group 1 to 6) in three semesters in primigravida and multigravida both was 13.95 ± 2.09 mmHg. (Table 5) Mean IOP of primigravida (Group 1,

3, 5) during whole period of pregnancy was 14.00 ± 1.87 mmHg and mean IOP of multigravida (Group 2, 4, 6) during whole period of pregnancy was 13.81 ± 1.08 mmHg. In puerperium the mean IOP in both primigravida and multigravida was 14.21 ± 2.33 mmHg. It showed IOP decreased more in multigravida than primigravida during whole antenatal period. Again IOP increased during puerperal period. IOP decreased during whole pregnancy period (both primigravida and multigravida combined) than control is statistically significant with p value < 0.0001 . IOP raised during puerperal period from IOP of whole pregnancy period of both primigravida and multigravida, whole primigravida pregnancy period and whole multigravida pregnancy period is statistically significant with p value < 0.0001 . Intraocular pressure variation in whole pregnancy period (group 1 to 6), primigravida pregnancy period (group 1,3,5), multigravida pregnancy period (group 2,4,6) and puerperal period (group 7,8) were compared with mean IOP of control group. Significant differences were observed with $p < 0.0001$.

4. Discussion

This cross-sectional study was conducted to find out relationship of IOP with pregnancy period. In this study IOP values decreased during pregnancy period from 1st trimester to 3rd trimester both in primigravida and multigravida and return to normal in puerperal period. Lowest IOP was seen in 3rd trimester of pregnancy. IOP variation during pregnancy and puerperium period was statistically significant. This decline of IOP is mostly temporary and comes towards normal level after delivery. Our study is consistent with other studies.³ Another study showed no significant effect of pregnancy on eyes with / without pre existing ocular diseases.¹¹ In pregnancy IOP decline gradually by 2-3 mmHg and this physiological decrease of IOP gradually comes to baseline non-pregnant condition towards 3rd month postpartum.^{1,8,9,14} It has been seen that IOP is lower in sitting position as done in Applanation tonometry and Non-contact method than Supine position as in Schoitz tonometry.[3] In our study control was 15.92 mmHg and healthy pregnant mother both primigravida and multigravida had 13.95 mmHg. Study by Ibraheem WA et al¹⁵ control group IOP was 14.24 mmHg and healthy pregnant mother had 13.24 mmHg.

The physiological mechanism of decrease of IOP during pregnancy is multifactorial.^{1,2} In pregnancy there is generalized decrease in peripheral vascular resistance.⁵ Decrease of IOP may be due to hormonal levels causing increase outflow, decrease scleral rigidity, decrease episcleral venous pressure and increase systemic acidosis without influencing aqueous humour production.^{5,7} Hormone progesterone released throughout pregnancy period cause decrease of scleral venous pressure there by reduces aqueous humor flow resistance causing decrease

Table 1: Demographic data of study groups: - Primigravida and Multigravida: Numbers, and age in trimesters of pregnancy and puerperium. Age range was 20 to 38 years. Primigravida (N-231) multigravida (N 231)

Categories	No. (%)	Mean Age \pm SD
Overall (Group 1 to 9)	693 (100%)	29.05 \pm 4.00
Study Group (Group 1 to 6)	462 (66.66%)	29.03 \pm 3.94
Group – 1 (1st Trimester Primi)	77 (11.1%)	27.02 \pm 3.54
Group – 2 (1st Trimester Multi)	77 (11.1%)	31.02 \pm 3.58
Group – 3 (2nd Trimester Primi)	77 (11.1%)	27.33 \pm 3.71
Group – 4 (2nd Trimester Multi)	77 (11.1%)	31.15 \pm 3.59
Group – 5 (3rd Trimester Primi)	77 (11.1%)	27.18 \pm 3.62
Group – 6 (3rd Trimester Multi)	77 (11.1%)	30.53 \pm 2.98
Group – 7 (Puerperium Primi)	77 (11.1%)	25.81 \pm 3.52
Group – 8 (Puerperium Multi)	77 (11.1%)	30.59 \pm 2.93
Group – 9 (Control Group)	77 (11.1%)	29.22 \pm 4.46

Table 2: Comparison of intraocular pressure of right and left eyes of primigravida, multigravida and puerperium of different study groups with control group

Categories	Range of IOP Min - Max	Right Eye IOP Mean \pm SD	Left Eye IOP Mean \pm SD	Both Eyes IOP Mean \pm SD	p value
Overall N 693 (Group 1 to 9)	08 - 20	—	—	14.19 \pm 2.19	—
Control / Non- Pregnant(Gr-9)	10 - 20	15.86 \pm 2.07	15.99 \pm 2.17	15.92 \pm 2.11	p = 0.7042 Not significant
Study Group (N-462) (Gr-1 to 6)	10 - 20	—	—	13.95 \pm 2.09	p < 0.0001 highly significant
1stTrimester Primigravida (Gr-1)	08 - 19	14.16 \pm 1.72	14.46 \pm 1.83	—	p < 0.0001 highly significant
1stTrimester Multigravida (Gr-2)	10 - 19	14.37 \pm 2.02	14.37 \pm 1.94	—	p < 0.0001 highly significant
2ndTrimester Primigravida (Gr-3)	10 - 19	14.64 \pm 2.11	15.00 \pm 2.08	—	p = 0.0044 highly significant
2ndTrimester Multigravida (Gr-4)	10 - 19	14.24 \pm 2.04	14.47 \pm 2.00	—	p < 0.0001 highly significant
3rdTrimester Primigravida (Gr-5)	10 - 16	12.88 \pm 1.77	12.84 \pm 1.73	—	p < 0.0001 highly significant
3rdTrimester Multigravida (Gr-6)	10 - 17	12.69 \pm 1.78	12.74 \pm 1.46	—	p < 0.0001 highly significant
Puerperium Total (N 154)	08 – 20	14.20 \pm 2.34	14.21 \pm 2.33	14.21 \pm 2.33	p < 0.0001 highly significant
Puerperium Primi (Gr-7)	08 – 20	14.55 \pm 1.97	14.55 \pm 1.82	—	—
Puerperium Multi (Gr-8)	08 – 19	14.02 \pm 2.50	14.06 \pm 2.55	—	—

Table 3: IOP variation in different trimesters and puerperium in comparison to control

IOP	1st trimester primi & multi n=154 (Gr-1 & 2) n1 = 77 n2 = 77	2nd trimester primi & multi n = 154 (Gr-3 & 4) n1 = 77 n2 = 77	3rd trimester primi & multi n = 154 (Gr-5 & 6) n1 = 77 n2 = 77	Puerperium primi & multi n = 154 (Gr-7 & 8) n1 = 77 n2 = 77	Control (G-9) n = 77
Mean \pm SD	14.34 \pm 1.87	14.58 \pm 2.06	12.79 \pm 1.69	14.21 \pm 2.33	15.92 \pm 2.11
Significance level and p value	Significant t = 5.796 d.f = 229 p < 0.0001	Significant t = 4.62 d.f = 229 p < 0.0001	Significant t = 12.19 d.f = 229 p < 0.0001	Significant t = 5.42 d.f = 229 p < 0.0001	

Table 4: Comparisons of intraocular pressure in primigravida, multigravida in different trimesters and puerperium with control

IOP	Group 1 n = 77	Group 2 n = 77	Group 3 n = 77	Group 4 n = 77	Group 5 n = 77	Group 6 n = 77	Group 7 n = 77	Group 8 n = 77	Group 9 n = 77
Mean \pm SD	14.31 \pm 1.78	14.38 \pm 1.97	14.82 \pm 2.09	14.36 \pm 2.01	12.86 \pm 1.75	12.71 \pm 1.63	14.55 \pm 1.88	14.04 \pm 2.52	15.92 \pm 2.11
Significance level and p value	Significant, t=5.12, d.f = 152, p < 0.0001	Significant, t=4.68, d.f = 152, p < 0.0001	Significant, t=3.25, d.f = 152, p < 0.0001	Significant, t=4.70, d.f = 152, p < 0.0001	Significant, t=9.79, d.f = 152, p < 0.0001	Significant, t=9.79, d.f = 152, p < 0.0001	Significant, t=10.56, d.f = 152, p < 0.0001	Significant, t=5.02, d.f = 152, p < 0.0001	

Table 5: IOP variation in pregnancy and puerperium

Category	Control (Gr- 9) Mean \pm SD n = 77	Whole Pregnancy period Primi & Multi (Gr-1 to 6) Mean \pm SD n = 462	Whole Pregnancy period Primigravida (Gr-1,3,5) Mean \pm SD n = 231	Whole Pregnancy period Multigravida (Gr-2,4,6) Mean \pm SD n = 231	Puerperial period (Gr-7,8) Mean \pm SD n = 154
IOP	15.92 \pm 2.11	13.95 \pm 2.09	14.00 \pm 1.87	13.81 \pm 1.08	14.21 \pm 2.33
Significance level and p value		Statistically significant, t = 764, d.f = 537, p < 0.0001	Statistically significant, t = 5.49, d.f = 306, p < 0.0001	Statistically significant, t = 11.34, d.f = 306, p < 0.0001	Statistically significant, t = 5.42, d.f = 229, p < 0.0001

of IOP. Its anti-glucocorticoid features may have a role of decreasing IOP. Otherwise Estrogen mediates for production of 2^{nd} messenger nitric oxide, prostacyclin, endothelin – 1 and ecosanoid vasodilators causing reduction of vascular resistance.^{2,10} This leads to vasodilator effect decreasing arterial pressure causing reduction in aqueous humour production. Aqueous humour production also decreases due to increase of beta human chorionic gonadotrophin acting on cyclic AMP.⁸ Endogenous corticosteroids have also ocular hypotensive effect. Relaxin also released in pregnancy that has softening properties decreasing corneal rigidity there by decrease aqueous humour production. Effect of relaxin on increase outflow facility is mediated by collagen changes there by decrease rigidity of schlemm canal and trabecular meshwork.¹³

In 1^{st} trimester IOP in primigravida 14.31 ± 1.78 mmHg and multigravida 14.38 ± 1.97 mmHg, there was no significant difference of IOP. In 2^{nd} trimester IOP in primigravida 14.82 ± 2.09 mmHg and multigravida 14.36 ± 2.01 mmHg and in 3^{rd} trimester IOP in primigravida 12.86 ± 1.75 mmHg and multigravida 12.71 ± 1.63 mmHg. There

is difference of IOP between primigravida and multigravida in all the trimesters of pregnancy. Ocular hypotensive effect in late pregnancy was more evident both in primigravida and multigravida and greater in multigravida. It is statistically significant. A study showed IOP in 1^{st} trimester was 16.4, 2^{nd} trimester 14.6 and 3^{rd} trimester was 12.2 which is statistically significant.² Other studies showed 3^{rd} trimester IOP is significantly lower than 1^{st} trimester and 2nd trimester.[3,10,13] In a study comparison of IOP of right eye was done in 3 trimesters showing lowest in 3^{rd} trimester.¹³ During puerperal period primigravida patients IOP was 14.55 ± 1.88 mmHg and in multigravida IOP was 14.04 ± 2.09 mmHg. IOP was significantly lower in multigravida than primigravida during antenatal period. IOP is higher during antenatal period in primigravida than multigravida in our study could be due to anxiety, stress and sleeplessness.^{5,16}

IOP in whole pregnancy period both in primigravida and multigravida together was 13.95 ± 2.09 mmHg; in primigravida was 14.00 ± 1.87 mmHg and in multigravida was 13.81 ± 1.08 mmHg. Our study control was 15.92

mmHg. Lower IOP during pregnancy period both in primigravida and multigravida than control coincides with the previous study.[15] IOP in multigravida is lower than primigravida is statistically significant. IOP in puerperium primigravida and multigravida combined was 14.21 ± 2.33 mmHg. This higher IOP in puerperal period than pregnancy period was statistically significant.

In this study the noteworthy finding was Ocular hypotensive effect during pregnancy and puerperium is significantly higher in multigravida than primigravida and more marked towards second half of pregnancy period. All the components of visual system undergo physiological changes during gestational period due to interaction of hormone, metabolic, immunological factors. Effect on ocular tissues may lead to exaggeration, resolution or improvement of disease conditions during course of pregnancy. So it is a very crucial step during pregnancy to monitor IOP in primigravida and multigravida as a part of antenatal examination. The pregnancy induced ocular hypotensive effect is beneficial to predisposing glaucoma more in multigravida than primigravida achieving the target IOP. Decrease of IOP more in multigravida than primigravida explains improvement of glaucoma. Although there is a narrow line of IOP changes between primigravida and multigravida. The exact reason of higher IOP in primigravida may be due to increasing pregnancy induced discomfort, anxiety, stress, sleeplessness and depression due to unusual variable physiological and anatomical changes creating psychological trauma. The multigravidas are more relaxed and relatively less tension as they are used to. Managing glaucoma during pregnancy and puerperal period poses significant difficulties. There is scarcity of studies in this sector. So regular prenatal IOP measurement including routine ocular examination should be carried out for continuous surveillance of ocular health.

Cessation of antiglaucoma medication is customized weighing risk benefit ratio in primigravida and multigravida. Avoiding antiglaucoma therapy to reduce IOP during pregnancy may be recommended for women who have had many pregnancies rather than first pregnancy. Pregnant women may be advised to cease taking antiglaucoma medication in order to mitigate the potential teratogenic impact on fetus; taking into account the ratio of risk to benefit. In primigravidas approach should weigh ocular benefit of treatment to mother against potential harm of antiglaucoma treatment on fetus. High risk glaucomatous individuals who cease glaucoma medication during pregnancy are susceptible to the progression of glaucomatous retinal damage. It is imperative to conduct vigilant surveillance of expectant mothers by ophthalmologist, obstetrician and pediatrician.

The limitation of our study was cross-sectional of a small sample size. It requires further study to establish the gravity of IOP difference in primigravida and multigravida

in different trimesters and puerperium. Further study also required to find out any hormonal differences in pregnancy period between primigravida and multigravida.

5. Conclusion

Systemic effect of pregnancy has an ocular component on decreasing IOP with variation in primigravida and multigravida in each trimester and puerperium. IOP decreased more in multigravida than primigravida which is more marked towards second and third trimesters. Higher IOP in primigravida could be due to pregnancy related stress. Cessation of antiglaucoma treatment usually advocated in multigravidas but in primigravidas should be different on giving benefit of anti-glaucoma treatment to mother against potential untoward effect on fetus. The decrease of IOP could be advantageous for preexisting glaucoma and other preexisting ocular conditions more in multigravida than primigravida. Therefore the physiological changes leading to decrease of IOP during antenatal and puerperal period should be in mind to prevent misdiagnosis and glaucoma management protocol.

Footnotes:-

Ethics: Institutional Ethics Committee approved.

Conflict of Interest: No conflict of interest was declared by the Authors.

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