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Biochemistry

A Study of estrogen as an endogenous diabetogenic factor in diabetic patients

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ABSTRACT

Aims & Objectives :
1) To estimate serum levels of estrogen in 30 normal healthy control subjects & 30 Type 1 & Type 2 Diabetes Mellitus (DM) patients. 2) To assess statistically whether estrogen is a most suitable parameter for prognosis & treatment of the DM patients.

Materials & Methods :
The present study was carried out in Department of Biochemistry, GMCH, Nagpur from May 2003 to May 2005. About 60 diabetic patients aged between 15-80 years were selected from diabetic OPD of GMCH, Nagpur under dept. of Medicine after written informed consent & after satisfying all inclusion criterias. 5 ml of fasting venous blood was withdrawn from each control & patient using a disposable syringe & needle under all aseptic precautions. The blood obtained thus was collected in a sterile bulb & allowed to clot at room temperature for at least 20 minutes. After this serum was separated by centrifugation. The serum thus obtained was used for the following estimations without further delay. All the water used in the following estimations was distilled & deionised & all reagents used were of analytical grade. The study subjects were classified into 3 groups : 1) Group I 30, controls, 2) Group II 30, type 1 DM patients, 3) Group III 30, type 2 DM patients. Serum estrogen levels in patients & controls were estimated using ELISA technique.

Results :
It is evident from the present study that levels of estrogen in female patients of Type 1 & Type 2 DM patients were significantly elevated as compared to controls.

Conclusion:
We may say that the determination of changes in estrogen can be considered as a valuable endogenous diabetogenic factor which result in abnormal glucose metabolism.

KEYWORDS : DM, ELISA

INTRODUCTION:

Estrogens are female sex hormones. They produce certain biological effects. They include 1. Growth of female genital organs, 2. The appearance of female secoundary sex characters, 3. Growth of mammary duct system & numerous other phenomenon, which vary somewhat in different species. Estrogen are produced in ovary by the maturing Graffian follicles, both theca cells & granulosa cells are involved & also in corpus luteum. All the three pituitary gonadotropins (FSH, LH & LTH) are involved in stimulation of estrogen secretion. Estrogens are also formed in adrenal cortex, placenta & testis in small amounts. The naturally occurring estrogens in humans are β – Estradiol, Estrone & Estriol. The principle estrogenic hormone in circulation & the most active form of estrogen is β – Estradiol, which is in metabolic equilibrium with estrone. Estriol is the principle estrogen found in the urine of pregnant women & in the placenta. After administration of estrogen there occurs proliferation of vaginal epithelium & endometrium, an increase in glycogen in vaginal epithelial cell, an increase in alkaline phosphatase activity in endometrium. There is increased rate of glycolysis with accumulation of lactic acid. Most of the effects of sex hormones on diabetes have been demonstrated in rats. Frequent administration of estrogen can develop a diabetogenic effect. The effect of estrogen on carbohydrate metabolism in diabetes was found by Houssay to vary markedly in different species. Estrogen treatment of rats soon after subtotal pancreatectomy increases incidence & severity of DM; while at a later stages estrogen caused amelioration of diabetes. Longer duration of estrogen use amoung current users may relate to increased risk of type 2 Dm[2].

AIMS & OBJECTIVES:

1) To estimate serum levels of estrogen in 30 normal healthy persons as controls & 30 Type 1 & 30 Type 2 DM subjects.
2) To assess statistically whether estrogen is the most suitable parameter for prognosis & treatment of patients.

MATERIALS & METHODS:

The present study was carried in Department of Biochemistry (DOB), of GMCH, Nagpur from May 2003 to May 2005. About 60 diabetic patients aged between 15-80 years were selected from diabetic OPD of GMCH, Nagpur under Department of Medicine after written informed consent & after satisfying all inclusion criterias.

INCLUSION CRITERIAS:

1) No h/o chronic diseases (HT, DM etc), 2) No past h/o TB, 3) Only nonsmokers & nonalcoholic patients were included.

We selected 30 healthy, normal volunteers with ages ranging from 15-80 years as controls. About 5 ml of fasting[3] venous blood was withdrawn from each control/patient using a disposable syringe & needles & under all aseptic precautions. The blood obtained thus was collected in a sterile bulb & allowed to clot at room temperature (RT) for at least 20 minutes. After this serum was separated by centrifugation. The serum thus obtained was used for the following estimations without further delay. All the water used in the following estimations was distilled & deionized & all reagents used were of analytical grade.

Study subjects were divided into 3 groups as follows :
1) Group (I) 30, controls, 2) Group (II) 30, type 1 DM patients, 3) Group (III) 30, type 2 DM patients.

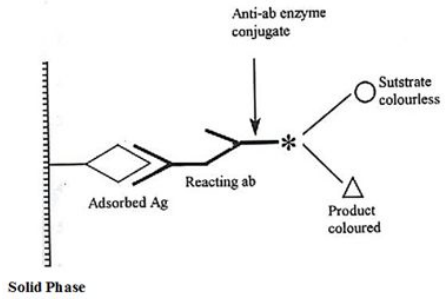
Methods of estimations:

Serum estrogen levels in patients & controls were estimated using ELISA technique.

Principle of ELISA[4]:

One of the immunoreagents is immobilized through adsorption on solid phase support (usually polyvinyl chloride or polystyrene) in such a way that there is no loss in its activity. The second immunoreagent is linked to an enzyme in such a way that there is no loss either to immunoreactivity or to enzyme activity.

After incubation & subsequent washing a chromogenic substrate of enzyme is supplied. If the two immunoreagents have bound to each other, colour will develop because of presence of linked enzyme. If there is no binding there will be no colour.



PRINCIPLE OF ELISA

Estimation of 17 β Estradiol in serum :

Principle : Anti –estradiol antibody (ab) are immobilized on microwell plate. Estradiol in sample competes with horseradish peroxidase (HRP) labeled estradiol for binding to the immobilized ab. After washing enzyme substrate is added , the amount of estradiol is inversely proportional to enzyme activity. Adding stop solution terminates the reaction. Absorbance is measured on a plate reader. The colour intensity is inversely proportional to estradiol concenteration in the sample.

Estradiol was estimated by Equipar diagnostic kit Preparation of reagents :

1) Diluted conjugate (Freshly prepared), 2) 10 µl conc. enzyme was mixed with 1 ml conjugate diluent.

Procedure :

All reagents, samples & Standards were brought to RT, atleast 1 hour prior to use.

Following addition were done as follows :

Reagents	Anti-estradiol IgG coated wells			
	A1 blank	B0	Stds	Samples
Conjugate diluent	-	100 µl	-	-
Stds	-	-	100 µl	-
Sample	-	-	-	50 µl
Control sample	-	-	-	50 µl
Conjugate	-	100 µl	100 µl	100 µl

Strips were covered with adhesive films. These were incubated at 37°C for 120 minutes. The film was peeled out & reaction solution was aspirated from all wells. Washed with 300 µl distilled water (DW). Washing procedure was repeated. Wells were striked on absorbent paper to remove water completely. 100 µl TMB substrate was added in each of the wells. All the wells were incubated for 30 minutes in dark. After that 100 µl of stop solution was added. Then absorbance was read of each wells E against A1 blanking well at 450 nm within 30 minutes.

E_2 reference values :

Women : 30 – 200 pg/ml,

Men : <40 pg/ml,

Children : < 60 pg/ml.

Statistical analysis : Data was analyzed on statistical software Intercostal stata version 7.

Continuous variables are presented as Mean _+ SD (Standard Deviation). Comparison between variables was done by using student –t-test . Analysis of variance (ANOVA) was used to see significant difference between variables. Categorical variables are represented in percentages. Categorical data was analyzed by using Chi-square test & p<0.05 was considered as statiscally significant.

OBSERVATIONS & RESULTS :

Table 1 : Distribution of study subjects according to gender

	Control	%	Type 1 DM	%	Type 2 DM	%
Male	10	33.33	10	33.33	10	33.33
Female	20	66.66	20	66.66	20	66.66
Total	30	100	30	100	30	100

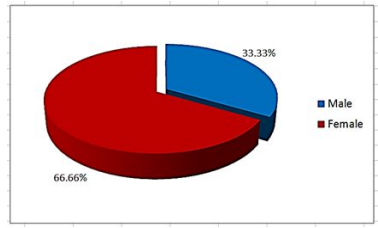


Table 2 : Estrogen values in sera of controls, type 1 DM & type 2 DM male patients

Sr. No.	Study groups	Mean ± S.D.	Range of values
1	CONTROL	32.27 ± 4.31	24 – 36
2	Type 1 DM	32.82 ± 5.12	25 - 37
		p>0.05	
3	Type 2 DM	34.29 ± 2.99	24 - 35
		p>0.05	

Estrogen values are expressed in pg/ml

Normal range given by Equipar diagnostic kit in males <60 pg/ml

Group I vs Group II – Non significant change

Group I vs Group III – Non significant change

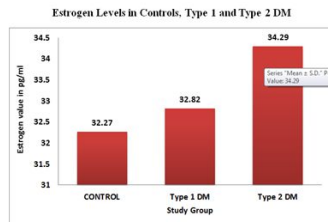


Table 3. Estrogen values in sera of control, Type 1 DM & Type 2 DM female patients

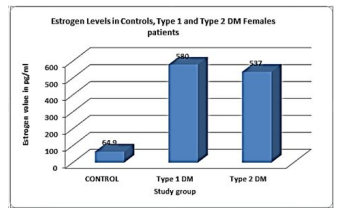
Sr. No.	Study groups	Mean ± S.D.	Range of values
1	CONTROL	64.9 ± 22.63	31 – 421
2	Type 1 DM	580 ± 30.82	350 – 690
		p<0.001	
3	Type 2 DM	537 ± 47.28	336 - 598
		p<0.001	

Estrogen values are expressed in pg/ml

Normal range given by Equipar diagnostic kit in females is 30-400 pg/ml

Group I vs Group II – Highly significant change

Group I vs Group III – Highly significant change



RESULTS :

Table 2 shows that there was no significant change in the findings of estrogen in type 1 & type 2 DM male patients as compared to normal controls.

While it is evident from table 3 that levels of estrogen in female patients of Type 1 & Type 2 DM were significantly increased as compared to controls.

DISCUSSION :

In the present study we found significant increase in estrogen level in female patients whereas there was no change in male patients as compared to controls. Hilal M[5], Lederer J[6], Willium M. Spellacy[7], studied the effect of contraceptives on carbohydrate metabolism. They found diabetogenic effect of estrogen. Similarly, estrogens and progestins used for contraception and hormone replacement therapy affect glucoregulation[8]. The relationship between metabolic syndrome, insulin resistance and estrogen is complex, and more research is needed to clarify the interplay of these hormones in health and disease[9]. There is a higher cardiovascular risk in diabetic women

than in nondiabetic women. This would suggest that women with diabetes do not have the cardioprotection associated with estrogen[10]. Much of the work has not been carried out in relation to our study regarding estrogen.

CONCLUSIONS :

During the course of the present study, we found that there was a significant increase in the levels of estrogen in females & no significant change in the levels of estrogen in males as compared to levels in normal healthy controls. In conclusion we may say that the determinations of changes in estrogen can be considered as valuable diabetogenic factor which result in abnormal glucose metabolism.

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