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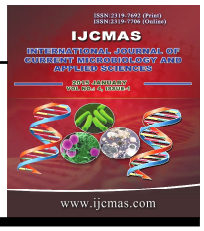

Original Research Article

Placenta and amniotic IgG and lysozyme: A monitoring of foetal damage of hypertensive or atherosclerotic mother mice

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Keywords

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A B S T R A C T

Foetal growth defects as a result of atherosclerosis and hypertension represent a major problem. Prediction of problematic of foetal disease as a result of metabolic disease led authors to detect a clinical investigation tool for assaying the criteria of foetal growth. In the present work, we assayed immunoglobulin G and lysozyme in placenta and amniotic fluid for observing the defect of growth. Our findings revealed that the placenta and amniotic fluid of 14 day old foetes of atherosclerotic with or without hypertension exhibited apparent depletion of immunoglobulin G and lyzozyme. These seemed to be interfered with reduction of the immune system of fetus and suspected defect during their growth. The authors concluded that pregnant mother should monitor the clinical investigation during pregnancy to aware of foetal growth defects.

Introduction

Intrauterine growth retardation (IUGR), which is defined as less than 10 percent of predicted fetal weight for gestational age, may result in significant fetal morbidity and mortality if not properly diagnosed (Vandenbosche and Kirchner, 1998). IgG represent the only antibody class that significantly crosses the human placenta. The uterine cavity contains innate immune detection and maintains sterility to protect the growing fetus (Marodi, 2006). In humans, nutrients and solutes that pass from maternal blood to fetal blood must traverse the histological barrier, which consists of two cell layers: the multinucleated syncytiotrophoblasts (STBs) and endothelial cells of the fetal capillaries. The fibroblasts and Hofbauer cells (i.e., placental macrophages) which are found in the villous stroma may be involved in the binding and trapping of immune complexes (Simister, 1998) which is essential for normal fetal growth and development. IgG transfer from

mother to fetus begins as early as 13 weeks of gestation and reached the highest peak in the third trimester (Saji *et al.,* 1999). Malek *et al.* (1996) reported increased level of IgG levels in the fetal circulation between 17 and 41 weeks of gestation.

The amniotic and allantoic fluids play a role in the immune protection of the fetus/newborn also in canine species (Dall'Ara, 2014). However, additional research is needed to better elucidate both the origin of IgG and lysozyme and the factors influencing the wide inter individual variations. The complex nature of amniotic fluid reflects contributions from many fetal systems, many functional roles, and multiple interactions with fetal maturation, obstetric, and maternal factors.

Normal amniotic fluid lysozyme content increased gradually from the early stage of gestation to the late part of the 2nd trimester, and rapidly after 32 weeks gestation. However, high levels of lysozyme activity were exhibited in cases of diabetic pregnancy, premature rupture of membrane, and fetuses with digestive disorders. However, amniotic fluid lysozyme content was low in cases of fetuses with anencephaly (Hisanaga *et al.,* 1982; Porto *et al.,* 1990). The levels of lysozyme were shown to be correlated with gestational age (Adinolfi *et al.,* 1976; Hagio, 1985).

Braunhut *et al.* (1984) detected maternal macrophage and numerous polymorphonuclear leukocytes in the intervillous spaces gave a positive reaction. Grigoriadis *et al.* (2013) reported that macrophages that are immature or lack the ability to be activated, such as the Hofbauer cell, may play an important role in the transmission of organisms capable to infect the placenta (like bacteria, chlamydia, toxoplasma, cytomegalovirus), as they have full phagocytosis capacity but may lack substances, such as lysozyme and peroxidase, necessary to destroy or inactivate the infectious agent.

There is no clear information about the exact role of IgG and lysozyme in placenta and amniotic fluid of mothers subjected to metabolic diseases with special interest for hypertension and atherosclerosis.

Materials and Methods

Induction of hypercholesterolemia

The experimental group was fed a hypercholesterolemic diet according to Enkhmaa *et al.* (2005). The hypercholesterolemic diet was composed of 3% cholesterol and 15% cocoa butter and 0.2% cholic acid and 0.2% thiouracil in accordance with the standard diet formula. The rats were fed for 6 weeks before the onset of gestation. The control group was supplied a standard diet free from atherogenic components.

Induction of hypertension

A healthy mouse with 25 g body weight was received subcutaneous infusion of angiotensin-II (0.7 mg/kg/day) for 16 week prior to conception according to Krege *et al.* (1995).

Experimental animal work

Eighty fertile male and virgin female rats of albino mice (*mus musculus)* (at a ratio of 1 male to 3 females) weighing approximately 25 g body weight, were obtained from Hellwan Breeding Farm, Ministry of Health, Egypt and used for experimentation. They were housed in cages with good ventilation on a 12-h light and dark cycle. Females were mated (1 male/3 females) overnight and zero

dates of gestation were determined the next morning by the presence of sperm in the vaginal smear. The pregnant mice were arranged into three groups (n = 20 per each) as follows: control; atherosclerotic (A) hypertensive; atherosclerotic and hypertensive. Animals were maintained in free excess of standard diet besides the formula of atherosclerosis and water *ad Libitum*. At the end of treatment, they were sacrificed by light diethyl ether anesthesia and dissected at 14th & 19th days prenatal and with placenta and amniotic fluids surrounding the fetuses were taken and processed for investigations of Immunoglobulin G (IgG) using the rat IgG ELISA kit (Alpha Diagnostic International, USA, Cat.No.6420) and lysozym**e** according to Schultz (1987). Unit of biologically active lysozyme was determined by using the following formula:

$$\text{Units of lysozyme/ml sample} = \frac{(\Delta A_{450nm}/\text{min Test} - \Delta A_{450nm}/\text{min Blank})(\text{df})}{(0.001)(0.1)}$$

Where, df is the dilution factor; 0.001 is the change in absorbance as per unit definition; 0.1 is the volume (in ml) of the sample used. At 19d-prenatal , placenta was separated and fixed in 10% formal saline, dehydrated in ascending grades ethyl alcohol, cleared in xylol and mounted in molten paraplast 58-62□C. Five m histological sections were cut and stained in haematoxylin and eosin.

Results and Discussion

Histological observations

In control 19d prenatal, the placenta is composed of the labyrinth zone, the basal zone, the decidua and the metrial glands. The basal zone is comprised of three types of differentiated cells, spongiotrophoblasts, trophoblastic giant cells and glycogen cells. The spongiotrophoblasts are present immediately above the trophoblastic giant cell layer located at the materno-fetal placental interface. The glycogen cells form multiple small cell masses and develop into glycogen cell islands in midgestation, and then most of them disappear before parturition. The decidua is comprised of the mesometrial decidual cells, and plays essential roles in the development of the vascularized decidual-placental interface. The cytoplasm of decidual cells at the placental side had numerous vacuoles, with larger cell type and few number smaller ones. Metrial cells were abundant in the decidualized myometrium. Myometrium and stroma contained large vacuolated cells with large nuclei and large vessels invaded by cytotrophoblasts and surrounded by numerous metrial cells. The cells in this layer shrunk and became more eosinophilic (Fig. 3A).

In 19d-prenatal of either atherosclerotic or hypertensive as well as of combined treatment, there was a detected of apparent placental necrosis appeared more commonly in the trophoblasts in the labyrinth zone. There was a detected degeneration and necrosis of trophoblasts and apoptosis of the spongiotrophoblasts in the basal zone. The decidualized myometrium revealed moderate karyorrhexis of decidual and metrial cells and larger vessels had neutrophilic granulocytic infiltration. Some small necrotic areas were also present. The trophospongium also contained inflammatory and necrotic cells (Figs. 3B–D).

From Figure 1 & 2, assaying immunoglobulin G and lysozyme in placenta and amniotic fluid of 14-dys prenatal revealed marked depletion in atheroclerotic with or without hypertensive groups. IgG and lysozyme showed marked

reduction in placenta and mother subjected to both hypertension and atheroclerosis.

From the present findings, the placenta and amniotic fluid of 14days old fetus of either atherosclerotic or hypertensive as well as in combined treatment, revealed apparent depletion of immunoglobulin G and lysozyme. These seemed to be interfering with reduction of the immune system of fetus and suspected defect during their growth. During fetal life, maternal immunoglobulin G (IgG) of the IgG1 subclass is delivered through the placenta to the fetus via interactions with the neonatal Fc receptor.

Fig.1 IgG contents (µM/100mg) of 14d-old placenta and amniotic fluid (AF) of atherosclerotic (A) mother alone or in combination with hypertension (A+H) showing significantly decreased lysozyme content comparing with control. Data are represented by the Mean±SE of 5 replicates (n=5), Significant at P< 0.05, C, Control; A, atherosclerotic; H, hypertensive; AH, Combined atherosclerotic and hypertensive group

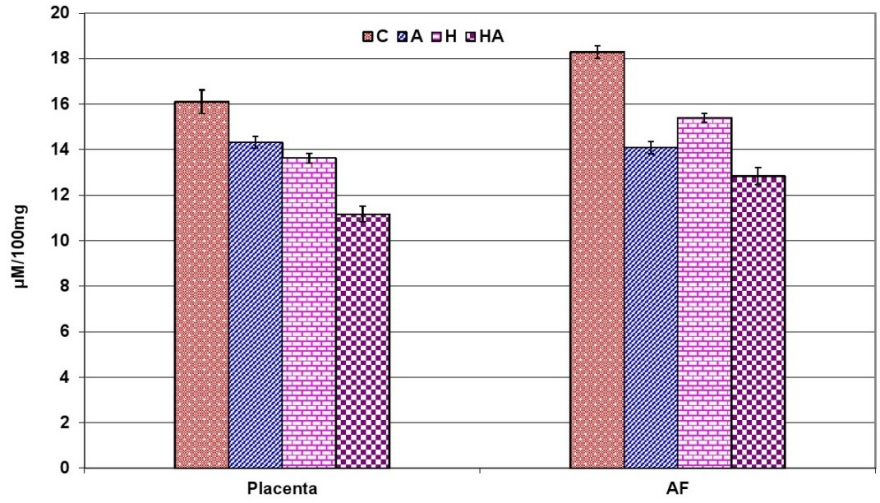


Fig.2 Lysozymes contents (µM/100mg) of 14d-old placenta and amniotic fluid (AF) of atherosclerotic (A) mother alone or in combination with hypertension (A+H) showing significantly decreased lysozyme content comparing with control. Data are represented by the Mean±SE of 5 replicates (n=5), Significant at P< 0.05, C, Control; A, atherosclerotic; H, hypertensive; AH, Combined atherosclerotic and hypertensive group

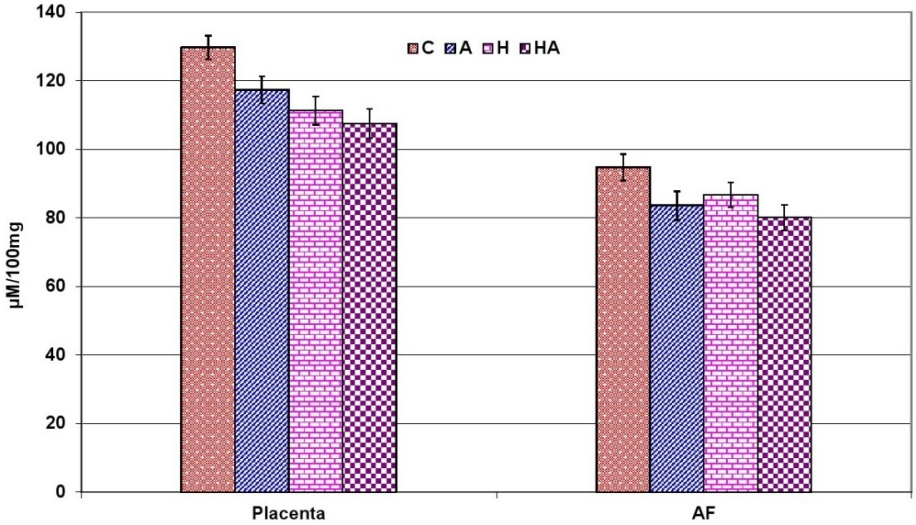
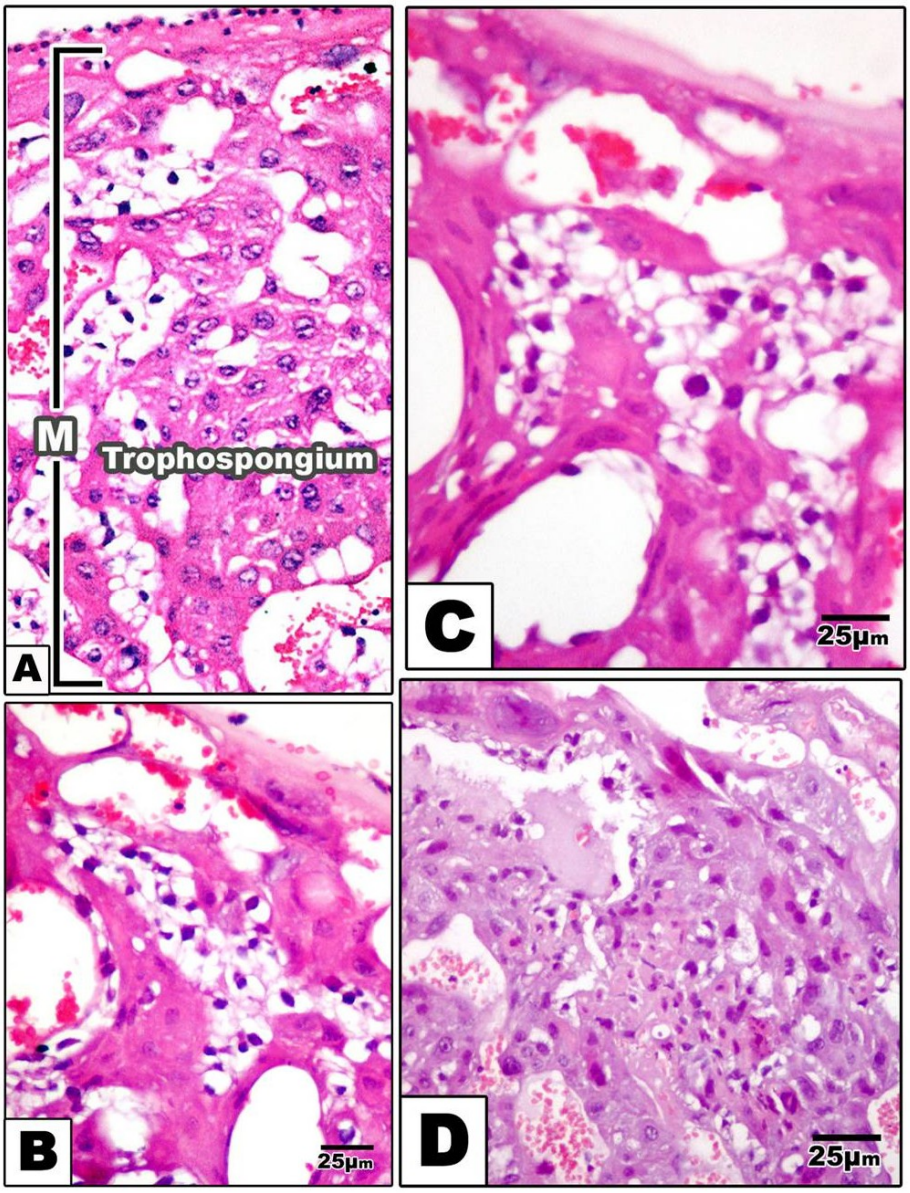


Fig.3A–C Photomicrographs of histological sections of placenta of 19days old mother mice. A. Control, B. Atherosclerotic, C. Hypertensive, D. Atherosclerotic and hypertensive, HX-E.



Transplacentally acquired maternal IgG is important for protection of infants in the early months of life from bacterial or viral infections. Although both systems of IgG transfer occur in humans and rodents, placental transfer is more efficient in humans (Roopenian and Akilesh, 2007). Also immunization of low-density lipoprotein-receptor-deficient (LDLR-/-)) female mice with OxLDL was shown to reduce the development of atherosclerotic lesions in susceptible LDLR)/) offspring (Yamashita *et al.,* 2006).

Low concentrations of IgG in cord blood were found in 3 conditions--the donor twin

in the fetofetal transfusion syndrome, hydrops fetalis and congenital hepatic disease. These were all associated with placental oedema. It is suggested that the oedema may be responsible for a disturbance in maternofetal placental transfer (Bryan, 1977).

Similar finding of depleted lyosozyme was detected in high-risk pregnant women with signs of fetal distress, regardless of neonate birth weight (Porto *et al.,* 1990). The lysozyme concentration in amniotic fluid increased gradually with gestational age, and showed apparent antibacterial effect against bacterial infection such as subtilis, *Staph. aureus* and *E. coli.* (Hagio, 1985) increasing the immune resistance of the developing fetus. Amniotic fluid lysozyme content was low in cases of fetuses with anencephaly (Hisanaga *et al.*, 1982).

On the other hand, the reduction of IgG reflects the pathological changes in the placenta of 14 days-old fetuses.

Similar findings were achieved by Chen *et al.* (1996) who mentioned that the immunostaining of IgG, IgE, C3, C4, and 5-HT were seen in the wall of villous vessels in pregnancy induced hypertension (PIH) patients, accompanied by aggregation of mast cells and lesions of villi and villous arterioles.

The authors concluded that IgG and lysozyme reduction in placenta and amniotic fluid is a monitoring of affected foetal tissue during prenatal life of hypertensive with or without atherosclerotic mother.

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