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Full Length Research Paper

Biochemical Changes of some Human Placental Enzymes and Nucleotides in Gestational Diabetic and Hypertensive Pregnant Women

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Abstract
Preeclampsia is a multisystem pregnancy complication, and representing about 2% to 8% of all pregnancies. It is characterized by high blood ATP level. The present study was carried out to determine changes of the activity levels 0f placental alkaline phosphatase (PALP), acid phosphatase (AP), and Na^+/K^+ ATPase in diabetic and hypertensive pregnant women to be used as a markers for the causes of preeclampsia. In this study, 18 human placentas were divided into three groups including normal, diabetic and hypertensive pregnancy cases. The activities of placental alkaline phosphatase, acid phosphatase, and Na^+/K^+ ATPase beside HPLC nucleotide analysis were determined in all studied placental groups. The results showed that, a marked decrease in ALP, AP and Na^+/K^+ ATPase activities were recorded in gestational hypertensive cases. Also, low activity levels of ALP and Na^+/K^+ ATPase were observed in gestational diabetic cases as well as elevated AP activity. The nucleotide HPLC analysis indicates decreased levels of mitochondrial ATP and GMP nucleotides with an elevated levels of UMP and some metabolites as adenine and adenosine in placentas of hypertensive cases. Meanwhile, increased CTP level and the appearance of lysosomal metabolites of the nucleotides were detected in the placentas of diabetic cases.

Keywords: *Human placenta, Preeclampsia, Placental alkaline phosphatase, Acid Phosphatase, Na/K ATP ase, nucleotides*

Introduction

The placenta constitutes the active interface between the maternal and fetal blood circulations and is responsible for a multitude of functions critical for fetal development, including nutrient transport, hormone production and providing an immunological barrier (Thomas and Theresa, 2013). High-risk pregnant women include those with uncontrolled diabetes, hypertension and pre-eclampsia (Silver, 2012) .

Human placental alkaline phosphatase (PALP) has been subjected to many studies because of its role in the transport mechanism and fetus development. It was suggested that alkaline phosphatase could be used as a parameter for measuring the placental functions (Mangal et al,2005). Serum from pregnant women in the last trimester of gestation shows activity of soluble placental alkaline phosphatase (sol-PLAP). It is also known that a membrane bound high molecular weight placental-ALP (high Mr-PALP) is present in butanol extracts from placental tissue. A developed method by Sembaj et al, (2000) allowed the detection of membrane-bound alkaline phosphatase in pellet of plasma centrifuged at 100,000x g. By applying this method they had detected a high molecular weight placental alkaline phosphatase in plasma of healthy pregnant women in the third trimester of pregnancy. In addition, there is an association between low molecular weight acid phosphatase (AP) and the degree of glycemic control via regulation of insulin – receptor signaling through phosphotyrosine pathways (Tok et al, 2006).

Na^+/K^+ ATPase is distributed to both the microvillus membrane and basal membrane of the placental syncytiotrophoblast is important in maintaining the electrochemical gradient for Na^+ ions, which represent the driving force of Na^+-coupled transport of nutrients (Jahansson et al, 2003). Regulation of placental nutrient transporters is of particular importance for the placental response to altered maternal nutrient supply. A large number of transporters for glucose, amino acids, fatty acids, ions, and micronutrients are expressed and active in the microvillus membrane and basal membrane (Lager and Powell, 2012). In pregnancy, there is an increased demand for energy and proteins to enable fetus and placenta to grow (Duggleby and Jackson, 2002). Adenosine is related to normal functioning of organs and tissues as in placenta (Agnes, 2005). This may be attributed to the vasodilatory effect of adenosine which may play a role in the hemodynamic changes in pregnancy (Escudero and Sobrevia,2012). During inflammation, hypoxia, or ischemia, ATP levels could be increased to 3-fold. This is also seen in diseases such as cystic fibrosis, chronic obstructive pulmonary disease, and preeclampsia (Mortaz et al, 2010). ATP may be hydrolysed faster during pregnancy as the ATP hydrolysing enzymes CD39 (capable of clearing atherosclerotic plaque) and alkaline phosphatase are highly expressed in the placenta. These pregnancy adaptations suggest that extracellular ATP levels need to be tightly regulated during pregnancy (McRae et al, 2013).

Therefore, in the current study, the alterations in some enzymatic parameters of the human placentas of gestational diabetic and hypertensive egyptian pregnant women were determined as well as the HPLC analysis of their nucleotide components.

Materials and Methods

Chemicals

ATP, ADP, AMP, adenine, adenosine and uracil (spectral grade), Tris hydroxmethyl aminomethane disodium salt, ouabain , bovine serum albumin and P-nitrophenol were purchased from Sigma Chemical Company. Sucrose and ethylenediamine tetraacetic acid (EDTA) from Merck. Ferrous sulfate, ammonium molybdate and Folin reagent from BDH Company. P-nitrophenyl phosphate disodium salt hexahydrate, ammonium phosphate, perchloric acid were purchased from Aldrich chemical company, (USA).

Human placenta samples

Eighteen human placentas from normal pregnant women (group1), diabetic pregnant women, already treated with daily insulin dose (20 U/ml), (group 2) and hypertensive pregnant women, already treated with acceptable oral antihypertensive drug under the supervision of specialist doctor (group 3), 6 placentas (average weight 480 gram) for each group were obtained with permission, at delivery, from pregnant women (average age, 22-31years) admitted to delivery unit (Shatby Maternity Hospital, Alexandria University) under the supervision of Dr.Sayed Albadawy, Professor of gynecology and obstetrics, Faculty of Medicine, Alexandria university. Immediately after delivery placenta was extensively washed with distilled water, followed by saline and kept in refrigerator at -20°c till used. The weight, diameter, and color of collected placentas, as well as weight and age of mother beside weight of fetus were shown in table (1).

Preparation of lysosomal, mitochondrial and microsomal fractions of human placentas

Placental tissue (80 gm) was homogenized with 150 ml Tris HCl- Sucrose- EDTA buffer (0.05M Tris HCl containing 0.32 M sucrose and 0.001 M EDTA, pH 7.4.) using a Woring Blender. The homogenate was centrifuged at 4000 rpm for 10 minutes using DAMON/ ice Division, CRU. 5000 centrifuge. The obtained pellets were discarded and the supernatant was recentrifuged at 10,000 rpm for 20 minutes at 4°C using MSE-Europa 65 ultra centrifuge. The sedinented lysosomal fraction was resuspended in 2ml 70 mM Tris-HCL buffer pH 7.4 containing 6mM KCL and 5 mM $MgCl_2$. The obtained supernatant was recentrifuged at 17,000 rpm for 20 minutes, at 4°C. The precipitated mitochondrial pellets were suspended in 2ml 70 mM Tris-HCL buffer pH 7.4, and the obtained supernatant was recentrifuged at 35000 rpm for 1 hr, in which the pellets of the precipitated microsomal fraction was in turn resuspended in 2 ml of Tris HCL buffer pH 7.4, according to the method of Peter and Bedrich (1980). The activities of alkaline phosphatase,acid phosphatase and Na+,K+-ATPase aswell as HPLC-analysis of the nucleotide components of each subcellular fraction were determined.

Estimation of protein

Protein concentrations were estimated according to the method of Lowry et al.(1951) using standard curve of bovine serum albumin with different concentrations, absorbance was measured at 750nm.

Assay of placental alkaline and acid phosphatase activities

Estimated according to the method of Bergmeyer (1963), using standard curve of P-nitrophenol, absorbance measured at 405nm. For alkaline phosphatase, the buffer substrate solution was made by dissolving 165mg of P-nitrophenyl phosphate in 0.05M glycine buffer pH 10.5. For acid phosphatase, the buffer substrate solution was made by dissolving 165mg of P-nitrophenyl phosphate in 0.05M citrate buffer pH 4.8.

Na^+/K^+ Adenosine triphosphatase activity

Determined as reported by Peter and Bedrich (1980), using standard curve of disodium hydrogen phosphate, absorbance was measured at 740nm. All the optical densities were measured using (Optima photo mech 301-D+) spectrophotometer.

Isolation of the nucleotides from the subcellular placental fractions

Some nucleotides, nucleosides and nitrogenous bases of lysosomal, mitochondrial and microsomal fractions were isolated from placentas of normal, diabetic and hypertensive pregnant women according to the method of Pimenove et al.(1988), separated and detected by high-performance liquid chromatography.

Reversed-phase HPLC analysis of the nucleotides, nuclosides and adenine

Some standard nucleotides (ATP, ADP, AMP and NAD), nucleosides (Adenosine and uracil), and a nitrogenous base (adenine) as well as the isolated nucleotide components from the subcellular fractions (50µL each) were applied to Peckman HPLC equipped with column of Hypersil C18 (150 mm × 5 µm × 4.6 mm), connected with UV spectra system detector. The analysis was carried out at a flow rate 1ml/min and chart speed 1 Cm / min. The mobile phase consisted of solvent gradient by mixing two solvents(A and B). Solvent A containing 10 mM potassium dihydrogen phosphate and 20 mM triethyl amine. Solvent B containing 20% acetonitrile in solvent A, pH 7.5. The column was equilibrated and eluted for 12 minutes with solvent A. After sample injection, the column was eluted for 10 minute with a linear gradient from 0 to 100% solvent A, then isocritically with solvent B for 10 min and with a revise gradient for 1 min. All solutions were filtered and degassed before use (Pimenove et al., 1988). The typical reversed-phase HPLC chromatogram of the separated standards is shown in figure (1).

Results

The weight, diameter, and color of collected placenta as well as weight and age of mother beside weight of fetus were recorded (Table 1).

Table 1: Weight of placenta, fetus, and mother, beside diameter and color of placenta.

Parameter	Group I	Group II	Group III
	Normal	Diabetic	hypertensive
Weight of placenta(g)	460-500	460-510	**450-490**
Diameter(cm)	14-15	18-19	**12-13**
Color of placenta	Red	Dark red	**Purple**
Weight of fetus(g)	3000-3200	3500-3700	**2900-3000**
Age of mother(yr)	20-27	22-31	**32-36**
Weight of mother(kg)	**75-79**	**85-90**	**74-78**

Alkaline and acid phosphatase activities of the subcellular placental fractions

The mean values of the specific activities (µmole P-nitrophenol/mg pr./hr) of ALP enzyme of lysosomal fractions (Table 2) were found to be 82267.3±10756.8 and 4039.8±631.7 for diabetic and hypertensive groups respectively compared to normal, 7163.3±414.6. While the mean values of enzyme activityes (µmole P-nitrophenol/mg pr./hr) of mitochondrial fractions were found to 6634.2±1054.89 and 1454.3±450.6 for diabetic and hypertensive groups respectively, compared to normal; 44368.7±5763.3. Also, the mean values of enzyme activity of microsomal fractions were, 1026.7±60.6 and 66.7±9.9 for diabetic and hypertensive groups respectively, compared to normal; 174.2±14.5.

The specific activities (µmole P-nitrophenol/mg pr./hr) of acid phosphatase (AP) of the above mentioned subcellular fractions were estimated (Table 2). The mean values of specific activities of lysosomal fractions were found to be 7.6±2.0 and 31.9±12.4 for diabetic and hypertensive groups respectively, compared to normal, 50.6±5.0. The mean values of specific activities (µmole P-nitrophenol/mg pr./hr) of mitochondrial fractions were found to be 8.1±2.6 and 7.0±0.8 for diabetic and hypertensive groups respectively compared to normal; 69.9±15.8. while the mean values of acid phosphatase specific activities (µmole P-nitrophenol/mg pr./hr) of microsomal fractions were 318.4±34.4 and 108.3±11.8 for diabetic and hypertensive groups respectively, compared to normal; 131.0±9.96.

Table 2. The measured enzymatic activities of lysosomal, mitochondrial and microsomal fractions in normal, diabetic and hypertensive pregnant women.

Pregnancy case		Alkaline phosphatase (µmole P-nitrophenol/mg pr./hr)			Acid phosphatase (µmole P-nitrophenol/mg pr./hr)			$Na^+ K^+$ ATPase (µmole Pi/mg pr./hr)		
		L	M	C	L	M	C	L	M	C
Normal	Mean ($\bar{\chi}$)	7163.3	44368.7	174.2	50.6	66.9	131.0	103.20	126.0	1394.8
	±S.D.	414,6	5763.3	14.5	5.0	15.8	9.96	16.60	30.5	202.7
	±S.E.	169.3	2352.9	5.93	2.0	6.5	4.07	6.77	12.4	82.8
Diabetic	Mean ($\bar{\chi}$)	82267.3	6634.2	1026.7	7.6	8.1	318.4	162.2	588.2	126.8
	±S.D.	17056.8	1054.8	60.6	2.0	2.6	34.4	17.47	228.1	22.0
	±S.E.	6963.4	4306.6	24.72	0.8	1.10	14.04	7.13	93.1	9.0
	P1	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	P2									
Hypertensive	Mean ($\bar{\chi}$)	4039.8	1545.3	66.7	31.9	7.0	108.3	84.2	91.9	339.1
	±S.D.	631.7	540.6	9.9	12.4	0.8	11.8	4.0	6.1	167.4
	±S.E.	257.9	184.0	4.05	5.6	0.3	4.82	1.63	2.5	68.3
	P1	0.591	<0.0001	<0.0001	<0.001	<0.0001	0.091	<0.034	0.663	<0.0001
	P2	<0.0001	<0.0001	<0.0001	<0.0001	0.849	<0.0001	<0.0001	<0.0001	<0.029

N: Normal L: Lysosomal S.E.: Standard error of the mean Unit: µmole Pi /mg pr./hr. D: Diabetic M: Mitochondrial S.D.: Standard deviation Specific activity: µmole Product/mg pr./hr. H: Hypertensive C: Microsomal

Na+/K+-ATPase activity of the subcellular placental fractions

The specific activity of Na^+/K^+ ATPase in each fraction was estimated (Table 2). The mean values of the enzyme specific activities of lysosomal fractions were found to be 162.2±17.47 and 84.2±4.03 µmole Pi/mg protein/hr for diabetic and hypertensive groups respectively, compared to normal, 103.2±16.6 µmole Pi/mg protein/hr. While, the mean values of enzyme specific activities of mitochondrial fractions were, 588.2±228.1 and 91.9±6.1µmole Pi/mg protein/hour for diabetic and hypertensive groups respectively, compared to normal, 126.0±30.5 µmole Pi/mg protein/hour. Also, the mean values of enzyme specific activities of microsomal fractions were, 126.8±22.0 and 339.1±167.4 µmole Pi/mg protein/hr for diabetic and hypertensive groups respectively, compared to normal; 1394.8±202.7 µmole Pi/mg protein/hr.

Reversed-phase HPLC analysis of the nucleotides, nuclosides and adenine of the subcellular placental fractions

The retention time of some standard nucleotides, nucleosides and adenine as a nitrogenous base were separated by high performance liquid chromatography (HPLC) (Figure 1). The concentration values of the separated nucleotide components in the subcellular fractions of placentas of diabetic and hypertensive women compared to normal are represented as their peak areas (%) and the total areas (%) of the their HPLC chromatogram (Table 3).

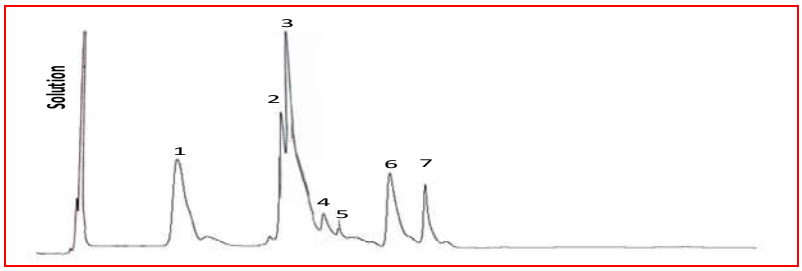

Fig 1: Typical reversed phase HPLC chromatogram of some standard nucleotides, nucleosides and nitrogenous bases.

1- Uracil (R.t. 10.93 min) 5- NAD (R.t. 17.20 min)
2- AMP (R.t. 13.22 min) 6- Adenine (R.t. 19.71 min)
3- ADP (R.t. 14.77 min) 7- Adenosine (R.t. 20.67 min)
4- ATP (R.t. 15.85 min

Discussion

The obtained results revealed that the activity of alkaline phosphatase (ALP) which isolated from the mitochondrial fraction of normal placenta was significantly higher ($p < 0.05$) than that of both lysosomal and microsomal fractions of normal placenta. This result was in agreement with Carl et al, (1996), who reported that the mitochondrial fraction exhibited the highest level of alkaline phosphatase activity, and that may attributed to the role of ALP in the oxidative phosphorylation process in mitochondria. In case of hypertensive pregnant women, the ALP activity has been appreciably decreased for the lysosomal, mitochondrial and microsomal fractions respectively compared to normal cases. This significantly lowered value in ALP activity ($p < 0.05$) may lead to weak hydrolysis of phosphate esters and consequently low deposition of calcium phosphate complex in fetus bones which may lead to unhealthy fetus and accordingly to a risk of foetal survival. This interpretation was found to be in agreement with that obtained by Williams (1991), who suggested that the lower activity of alkaline phosphatase may interpret the weak metabolic activity in hypertensive placenta and the risk of foetal survival. Johnstone et al.(2005) reported that the failure of placental trophoblasts to differentiate in case of hypertensive pregnant women leads to a decrease in expression of placental alkaline phosphatase and that leads to inhibition of adenylate cyclase enzyme and reduced production of intracellular c-AMP.

On the other hand, diabetes is amplified during pregnancy, resulting in a higher incidence of adverse pregnancy outcomes such as pre-eclampsia and placental insufficiency, as reported by Christin and Mary (2001). Our study revealed that, ALP activity in the mitochondrial fraction of the placenta of diabetic pregnant women showed a significant lower value compared to normal cases ($p<0.05$). That result was agreed with that previously obtained by Koyama et al.(1998), who found that the lower level of serum alkaline phosphatase activity in patients with diabetes mellitus apparently originates from the selective disappearance or decrease in bone alkaline phosphatase activity in the circulation. However, the obtained result ensured that the risk of foetal survival still exist in diabetic pregnant women but with lower risk compared to that of hypertensive cases, and showed more probability of a well improved fetus bone formation than hypertensive cases.

Thomas (2001), showed that diabetes in pregnancy is associated with an increased activity of syncytiotrophoblast microvillous plasma membrane and that change results in an increased uptake of neutral amino acids across that membranes, which may be used in placental metabolism or may be delivered to the fetus. Since insulin is the primary growth promoting hormone in fetal life, and amino acids are more potent stimulators of fetal insulin release than glucose, so this change in amino acids delivery to the fetus may have profound effects on fetal growth rate. That may contribute to accelerated fetal growth in diabetic patients compared to hypertensive or normal cases. Moreover, determination of acid phosphatase activity in the lysosomal, mitochondrial and microsomal fractions isolated from the placental of hypertensive pregnant women showed a lower value compared to normal cases. Meanwhile the microsomal acid phosphatase in case of diabetic placenta revealed a significantly higher value compared to normal, while a significant decrease were marked in both lysosomal and mitochondrial fractions of diabetic women placenta. Previous data reported by Gloria et al. (1996), showed that there is an association between low molecular weight acid phosphatase (a member of the protein-tyrosine phosphatase family) and the degree of glycemic control., science, AP plays an essential role in the control of receptor signalling through phosphotyrosine pathways. Also, he found a significant association between the presence of acid phosphatase encoding gene (ACP_1) and the glycemic level in the last trimester of diabetic pregnant women. That quantitative variations of ACP_1 gene may influence the clinical manifestation of diabetic disorders and clarify the role of the acid phosphatase enzyme in the modulation of insulin receptor phosphotyrosine pathway. In addition acid phosphatase may play another important role in the appearance of gestational diabetic condition via the phosphorylation of insulin receptor substrate IRS-1,(Ramzi et al. 1994) and that was emphasized the published data by Tok et al. (2006), they reported that insulin receptor substrate (IRS-1) expression and tyrosine phosphorylation is decreased during pregnancy of gestational diabetic pregnant women. All these observations were found to be closed with our obtained results which pointed to the highest activity of the microsomal acid phosphatase in the gestational diabetic placenta.

Table 3: Retention time and area percent of nucleotides, nucleosides and a nitrogenous base of subcellular fractions of normal, diabetic and hypertensive placentas by high - performance liquid chromatography (HPLC).

Area	fraction	Peak No.	1	2	3	4	5	6	7	8	9	10	11	12	13
		Retention Time (min)	4.60	5.75	7.03	7.58	8.66	10.94	13.22	13.79	14.77	15.85	17.20	19.71	20.6713
		Neucleotide	CMP	UMP	GMP	CTP	CDP	Uracil	AMP	UTP	ADP	ATP	NAD	Adenine	Adenosine
Area %	L	Normal	13.87	3.72	2.31	--	5,83	1.51	2.40	2.04	3.21	9.11	Trace	Trace	Trace
	M		6.69	Trace	11.45	--	6.22	Trace	3.40	4.98	6.73	11.66	1.93	0.55	0.63
	C		7.95	Trace	1.34	--	10.97	0.22	1,40	Trace	3.51	2.34	2.61	0.99	Trace
Total area %			28.51	3.72	15.10	--	23.02	1.73	7.29	7.02	13.45	23.11	4.54	1.54	0.64
Area %	L	Diabetic	--	--	--	--	--	--	--	--	--	--	--	60.03	11.53
	M		0.31	0.72	0.62	--	--	--	--	--	1.63	2.65	1.03	--	--
	C		--	1.70	--	21.31	--	--	--	--	--	12.80	--	--	--
Total area %			0.31	2.2	0.63	21.32	--	--	--	--	1.64	15.54	1.03	60.04	11.53
Area %	L	Hypertensive	0.27	1.63	0.59	--	0.29	--	o.57	--	1.00	Trace	0.48	--	0.85
	M		4.73	0.26	1.98	--	14.59	0.86	3.49	--	3.98	2.46	--	1.33	--
	C		4.64	14,48	2.91	--	0.21	--	2.43	--	3.27	--	1.23	--	--
Total area %			9.64	16.42	5.48	--	15.09	0.87	6.50	--	8.25	2.46	1.71	1.33	0.85

L: Lysosomal M: Mitochondrial C: Microsomal

Moreover, Na^+/K^+ ATPase activity in the microsomal fraction of normal case was exhibited a higher value than that of the lysosomal and mitochondrial fractions. This agreed with the measured Na^+/K^+ ATPase activity isolated from the placenta of hypertensive pregnant women but with a lower value than that of the normal, this was confirmed by Bingini et al. (1995), they found a decrease in Na^+/K^+ ATPase activity in syncytiotrophoblast plasma membrane obtained from pregnant hypertensive women. Assuming the presence of an endogenous digitalis-like factor (ouabian), the results suggest a simple way of explaining the lower Na^+/K^+ ATPase activity in the placental membranes of hypertension (Amler et al., 1994) , that confirmed some structural change in the Na^+/K^+ ATPase. These changes might impair the function of Na^+ coupled transporters and contribute to the reduced growth of the fetus (Jahansson et al., 2003).

Furthermore, Na^+/K^+ ATPase activity corresponds to the microsomal fraction of diabetic pregnant women showed a significantly ($p < 0.05$) reduced value compared to normal. That result coincides with Zolese et al.(1997) they suggested that the reduced activity of Na^+/K^+ ATPase separated from the microsomal fraction of the placenta of diabetic pregnant women was attributed to a modification in the adenosine triphosphate binding site of the enzyme rather than decreasing number of active molecules.

However, the results obtained from the study on lysosomal and mitochondrial fractions of the diabetic cases showed a marked increased of Na^+/K^+ ATPase activity and that probably attributed to the injected insulin which may activate Na^+/K^+ ATPase bounded to cell membranes. Sibley et al. (1997), confirmed the result, suggesting that insulin causes K^+ to enter cells and the intracellular arise of K^+ may slightly activates Na^+/K^+ ATPase.

On the other hand, high performance liquid chromatographic analysis of the isolated placental purine, pyrimidine nucleotides beside some of their metabolites revealed the presence of purine and pyrimidine nucleotides in the three examined fractions of the placenta, in which the purine nucleotides were appeared with a higher concentration in the lysosomal and mitochondrial fractions, while pyrimidine nucleotides appeared in the microsomal fraction with more concentration than the other ones; may be due to their lower molecular weight, as well as the microsomal fraction were enriched with t-RNA and m-RNA concerning with protein biosynthesis process. Our study demonstrated that healthy normal placenta contains ATP with total peak area % to about 23.11 as a major purine nucleotide. That result was found to be agreed with very recently published data by Houghton (2006), who found that the trophectoderm layer which represents two thirds of the trophoblast cells consumed significantly more oxygen, produced more ATP and contained a greater number of mitochondria, and the major fate of the energy produced by this layer is likely to be the Na^+/K^+ ATPase which located on the trophectoderm basolateral membrane.

Comparison between healthy pregnant and hypertensive women demonstrated a trace amount of ATP to about 2.46 as total area %. That result probably owing to the release of ATP to the extracellular compartment as observed in cases such as inflammation, shear stress, and placental ischemia. That was in agreement with published data by Kadereit et al. (2005) they found that hypertension and sodium retention are features of a diminished 11B-hydroxy steroid dehydrogenase type 2 which is believed to play a key role in fetal development since this enzyme protects the fetus from exposure to high levels of maternal cortisol by virtue of converting maternal cortisol diseases states to its inert metabolic cortisone. Driver et al. (2003) postulated that 11β-HSD was proposed in regulating fetal growth and developing by protecting the fetus from maternal hypercortisolaemia. He reported a reduced enzyme activity in various diseases with abnormal renal sodium retention and hypertension including preeclampsia in which the released ATP causing a down regulation of that enzyme. This released ATP may inhibit gene expression of 11β-hydroxy steroid dehydrogenase type 2 via purinergic receptor which inhibits the expression and activity of that enzyme by a post transcriptional mechanism. , increased plasma levels of extracellular

The abnormal ATP level is considered to contribute to the development of the disease, since extracellular ATP has been shown to be a danger signal in many diseases. Extracellular ATP may increase blood pressure and activate endothelial cells and immune cells. In the circulation, ATP can be dephosphorylated to adenosine, which counteracts the effects of ATP (Harry et al., 2014).
In addition, apoptosis has been implicated in complication of pregnancy such as preeclampsia. Dash et al. (2003), suggested that the apoptosis of human extravillous trophoblast can be regulated by nitric oxide, and the anti-apoptic effects of nitric oxide in these extra villous trophoblast cells appear to be mediated through the production of c-GMP. These data were confirmed by Chakraborty et al. (2006), suggested that nitric oxide is an important cellular mediator of tissue repair as it is produced in macrophages by the enzyme inducible nitric oxide synthatase during wound healing, and he also found that the aqueous extract of human placenta can be used as a wound healer. That data was found to be coincides with our obtained results which revealed the presence of GMP in the healthy placental extract as a second major purine nucleotide with total area % equals to 15.10. Meanwhile, the obtained corresponding data from the hypertensive placental extract showed a significant decreased value to about 5.48 as total area %. That result may explain the atrophy of hypertensive placenta and consequently the risk of fetal survival. Moreover, appropriate regulation of ion transport by human placental syncytiotrophoblast is important for fetal growth throughout pregnancy. That ion transport can be modulated by extracellular nucleotide that raises the intracellular calcium via activation of the purinergic receptors. Roberts et al. (2006), proposed that the extracellular nucleotides such as UTP, ADP and ATP were activate this receptor and subsequently elevate the intracellular calcium which modulates syncytiotrophoblast homeostasis and/or maternofetal ion exchange.

Our obtained results indicated the presence of UTP and ADP nucleotides of normal placental extract with total area % equal to 7.02 and 13.45 respectively while their corresponding values for the hypertensive placenta were significantly decreased. That result was in agreement with the previously published data which emphasized the down regulation of the purinergic receptors and subsequently the release of intracellular calcium which affect the syncytiotrophoblast cells. That explanation was emphasized by the appearance of UMP nucleotide in the hypertensive placental extract with relatively higher value equal to 16.42 than the corresponding ones in normal placental extract, 3.72 as area % that probably attributed to the hydrolysis of UTP into UMP. In addition, CMP and CDP nucleotides were identified in normal placental extract with total area % equals to 28.51 and 23.02, while their corresponding values of hypertensive and diabetic placenta were found to be 9.64, 15.09, 0.311 and trace amount respectively.

Consequently, this result may explain the presence of the probably CTP nucleotide in the diabetic placenta with a distinct value equals to 21.32 as area %. That obtained data was found to be coincide already published data, which indicated the role of CTP as a nucleotide influence the ability of the insulin receptor to bind insulin by mimicking the action of ATP on the purinergic receptors and that leads to the appearance of gestational diabetes (Trischitta et al.,1984). Also, the appearance of low level of GMP, ADP and ATP nucleotides in the diabetic placental extract compared to normal placenta were explained the presence of their metabolites adenine adenosine and unidentified metabolite may be (guanine or guanosine) in the lysosomal fraction, that may be attributed to the presence of the specific purine, hydrolyzing enzyme with high activity in the diabetic placenta. Similarly, the appearance of adenine and adenosine metabolite in the hypertensive placental extract indicated the enhancement of the breakdown of ATP as a result of oxygen deficiency in the ischemic cases and the accompanied vasodilatation and increasing of the blood flow (Maguire et al., 1992).

Conclusion

The biochemical changes in gestational hypertensive pregnant women, showed a decrease in ALP activity which leads to weak placental transport and low deposition of calcium in fetus bones. Also, a decrease in Na^+/K^+ ATPase leads to impair the function of Na^+ coupled transport of nutrient and that contribute to the reduced growth of fetus. Beside a highly decreased level of mitochondrial ATP which may inhibit the expression of 11β-HSD type 2 enzyme, in turn leads to sodium retention associated the gestational hypertension. Meanwhile, Low level of ALP In gestational diabetic pregnant women indicated that the risk of fetal survival still exists but with a lower degree compared to hypertensive cases. Also, there was an increase in the activity of microsomal ACP which plays an important role in the control of insulin receptor signaling through dephosphorylation of IRS and phosphotyrosine residue of the receptor. Mitochondrial and lysosomal ATPase activities were slightly increased compared to normal, this elevation may be due to the injecting insulin which raise the intracellular K+, and that activate Na+/K+ ATPase.

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