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Research article

Impairment of thyroid and prostate in experimentally induced diabetes and atherosclerosis male Wistar albino rats

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Abstract

Prostate gland represents one of the main parts of male reproductive system. The influence of diabetes and or hypercholesterolemia on prostate damage is still not clear. In this experiment, forty male Wistar albino rats weighing approximately 100 g were arranged into four groups: control, diabetic (single i.p. 40 mg streptozotocin/kg B.wt) and hypercholesterolemic (diet supplement 3% cholesterol) and combined treatment. Treatment was carried out for 12 weeks. At the end of treatment, animals were sacrificed and prostate and thyroid gland were separated and processed for histological investigations, assessment of biochemical markers of vascular endothelial growth factors (VEGF), heat shock protein 70 (HSP-70), 8-hydroxy-deoxyguanosine (8-HDG), adhesive molecules ((ICAM-1 and VCAM-1) as well as quantitative and qualitative isoenzyme electrophoresis of malate dehydrogenase (MDH), lactic dehydrogenase (LDH), Aspartate aminotransferase(AST) and glucose-6 phosphate dehydrogenase (G6PDH). Comet assay for prostate was also determined. The present findings reported increased histopathological alterations in thyroid and prostate glands coincides with increased prostate levels of HSP 70, 8-HDG and activity of LDH, MDH and G6-PDH in comparison with the control. However, there was a marked depletion of prostate contents of VEGF, adhesive molecules (V-CAM, I-CAM) as well as the enzyme activity of AST. The assayed isoenzymes were markedly altered in diabetic and or hypercholesterolemic reflecting altered prostate function. These findings were parallel with increased DNA damage. It was concluded that diabetes and or hypercholesterolemia led to marked alteration of thyroid gland and interfered in prostate gland function as assessed by biochemical markers, comet assay and isoenzyme electrophoresis.

Keywords: Prostate gland; thyroid gland; diabetes; atherosclerosis

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Introduction

The incidence of diabetes, in particular type 2 diabetes, is increasing at an alarming rate. Worldwide, about 124 million people had diabetes by 1997; by 2010, this number was estimated to reach 221 million (Peppa et al., 2003). Diabetes and hypercholesterolemia have been closely associated with cardiovascular defects (McGarry, 1992; Black et al., 1998). Both diseases are associated with body organs complications such as myocardial disease (El-Sayyad et al., 2012a), skin (El-Sayyad et al., 2012b), ocular (El-Sayyad et al.,

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2011; El- Sayyad et al., 2015a), liver (El-Sayyad et al., 2014), bone (El-Sayy et al., 2015b) and brain disorders (El-Sayyad et al., 2015). Recently several studies have been reported showing close association between diabetes (Rabijewski et al., 2014), hypercholesterolemia (Dupont et al., 2014) and testosterone level.

In mammals, the prostate gland (tubuloalveolar exocrine gland) is a main component of the male reproductive system which serves for secretion of 30% of seminal fluid and its smooth muscles serve for erection (Cappelleri and Rosen, 2005). Erectile dysfunction is one of the most complication and major reason of decreased quality of life during and after various surgical treatment of prostate cancer (Alba and Wang, 2012; Casey et al., 2012).

Recently, research workers are looking for the relationship of diabetes and obesity with cancer of prostate. Metabolic syndromes such as abdominal obesity, hypercholesterolemia, hypertension, and diabetes have been associated with increased risk of prostate cancer (Pelucchi et al., 2011). Increased incidence of coronary artery disease (CAD) in patients with conventional cardiovascular risk factors was markedly associated with increased level of marker of prostatitis (Boroumand et al., 2011). Prostate cancer has also been associated with high blood-cholesterol level (Krycer and Brown, 2013).

The present work illustrates the effect of diabetes and hypercholesterolemia on prostate and thyroid gland structure.

Materials and Methods

Experimental work

Forty fertile male albino rats weighing approximately 100g were obtained from Breeding Farm, Ministry of Health, Giza, Egypt. They were fed on standard diet and water was allowed *ad libitum* throughout the experimental period. They were housed in good ventilation with 12 hour light and dark cycle. Male albino rats were fed on hypercholesterolemic diet for 12 weeks. Diabetes was carried out and animals were allowed on standard diet for the similar period of cholesterol fed rats. Rats were arranged into four groups such as control (C), hypercholestero-lemic (H), diabetic (D) and combination of hypercholesterolemic and diabetic (HD). Each group had 10 male rats.

Induction of hypercholesterolemia

The experimental group was fed a hypercholesterolemic diet as described by Enkhmaa et al. (2005). The hypercholesterolemic diet was composed of 3% cholesterol and 15% cocoa butter, 0.2% cholic acid and 0.2% thiouracil. The diet was fed for 12 weeks. The control group was supplied a standard diet free from atherogenic components.

Induction of diabetes

Experimental diabetes was induced in all the rats (n=10) by a single interperitoneal injection of streptozotocin (40 mg/kg) in citrate buffer (0.05 M) (pH 4.5) (Povoski et al., 1993) and fed on standard diet for 12 weeks. Control animals were treated with physiological saline as vehicle. Hyperglycemia was verified by measuring the blood glucose more than 200-240 mg/dl.

Experimental animal work

At the end of treatment, male rats of both control and experimental groups were sacrificed and dissected. The prostate and thyroid glands were subjected for the following investigations.

Histological investigation

At the end of treatment, experimental groups were euthanized using choloroform and dissected. The prostate and thyroid glands of both control and experimental groups were separated. They were fixed in 10% phosphate buffered formalin (pH 7.4), dehydrated in ascending grades of ethyl alcohol, cleared in xylene and mounted on molten paraplast at 58-62ºC. Five µm histological sections were cut, stained with Hematoxylin & eosin and investigated under a bright field microscope.

Biochemical assays of prostate gland

Vascular endothelial growth factor (VEGF)

ELISA-plate coated with VEGF165 monoclonal antibody (R&D System, Minneapolis, MN, USA) was used and assayed according to manufacturer instructions. Absorbance was measured at 450 nm and 540 nm by an ELISA plate reader UV-Spectrophotometer (Perkin Elmer Victor 3™) with Software Wallac 1420 version 3.0.

Heat shock protein (Hsp 70)

Hsp 70 was measured by an ELISA immunosorbent assay (Nunc Immunoplate Maxisorp; Life Technologies,UK). The absorbance was read at 492 nm by using a plate reader with Genesis 2 Software (Life Sciences, Basingstoke, and Hampshire, UK) (Xu et al., 1993).

8-hydroxy-2-deoxy guanosine (8-OH-dG)

The concentration of 8-OH-dG was determined using the Bioxytech 8-OHdG-ELISA Kit (OXIS Health Products, Portland, OR, USA) according to the manufacturer's instructions. The absorbance was measured using a FLUO star OMEGA microplate reader (BMG LABTECH Ltd., Germany) at a wavelength of 450 nm (Attia, 2012). Reported values are the average of triplicate determination and tissue levels of 8-OHdG are expressed as ng/ml.

Determination of adhesive molecules (ICAM-1 and VCAM-1)

Prostate tissue was frozen at 80°C until measurement of adhesive molecules with a commercially available enzyme- linked immunosorbent assay kit (R&D Systems; Minneapolis, MN) following protocols provided by the manufacturer.

Maleic dehydrogenase (MDH)

MDH (EC 1.1.1.37) is a mitochondrial enzyme which reversibly converts L-malate into oxaloacetate in the presence of NAD. The enzyme was determined with either NAD or NADP as coenzyme (Wolfe and Neilands, 1956). In the reverse direction, the decrease in extinction at 340 nm was followed in a cuvette containing 2.7 ml of 0.1 M phosphate buffer (pH 7.0), 0.1 ml of reduced NAD (NADH) or reduced NADP (NADPH; 3 mg/ml) 0.1 ml of extract, and 0.1 ml of 20 mm oxaloacetate. Particulate malate oxidase was assayed by using 2,6-dichlorophenol-indophenol as electron acceptor and following the decrease in extinction at 600 nm.

Lactic dehydrogenase (LDH)

LDH was determined according to the method of Beebee and Carty (1983). Briefly, the assay depends on the reduction of NAD + to NADH and a change in absorbance at 340 nm which is proportional to the concentration of lactate in the medium. A standard curve was constructed using lactate concentrations ranging from (0–1000 mM lactate).

Aspartate aminotransferase (AST)

AST was assayed according to the method of Itoh and Srere (1970) with spectroscopic method. Oxaloacetate produced by GOT from aspartate was condensed with acetyl CoA to form citrate and CoA in a system coupled with citrate synthase. The CoASH (reduced coenzyme A) formed was measured by its reaction with DTNB (5,5'-dithio-bis (2-nitrobenzoic acid). The reaction was measured at 412 nm.

Glucose -6 phosphate dehydrogenase (G6PD)

The reaction velocity was determined by measuring the increase in absorbance at 340 nm resulting from the reduction of NAD or NADP. One unit reduces one micromole of pyridine nucleotide per minute at 30°C and pH 7.8 under the specified conditions. G6PD activity was determined in a continuous assay by monitoring the reduction of NADP+. Standard reaction mixtures contained in a volume of 1 ml, 50 µmol Tris-HCl (pH 8.3 or pH 7.2), 2.5 µmol glucose-6-P, 0.2 umol NADP+, 25% (v/v) glycerol, and an appropriate amount of the enzyme (Donohue et al., 1981).

Isoenzyme electrophoresis of esterase (EST), G6PD, LDH and MDH

Prostate of both control and experimental groups were cleaned and homogenized using 0.1 M Tris-HCl (pH 7.5) containing 20% sucrose and their protein content was determined according to Lowry et al. (1951) and electrophoresis was carried out according to Laemmli (1970). The protein bands were stained with Coomassie blue R-250 (60 mg/l) in an acidic medium (Andrews, 1986). For visualization of the tested enzymes, electrophoresis of lens tissues were carried out in the selected incubated medium for each kind of the assessed enzyme as follow.

Esterase

Esterase isoenzyme was estimated according to López-Soler et al. (2008). The gel was stained for esterase activity for 2-12 h at 4°C in 100 ml a-naphthyl acetate/fast blue solution in 0.2 M-phosphate buffer at pH 7.5. The resulting gel was measured and intensity of band staining was noted. The esterase isoenzyme data for each isolate are based on at least two replicate extractions, each duplicated on a single gel.

G6PD

Gel electrophoresis was performed at 4°C in polyacrylamide slabs using a method based on Gaal et al. (1980). The stacking gel (2.8% acrylamide) was prepared in 50 mm Tris-Pi (pH 6.3) and 20% sucrose and the separating gel (5% acrylamide) in 0.75 M Tris-Pi (pH 8). The electrophoretic buffer contained 5 mM Tris, 80 mm aspartate, and 20 µM NADP+ at pH 7.4. Gels were stained for G6PD activity at 30°C in a solution in a volume of 20 ml 1.2 mmol Tris-Pi (pH 8.5), 25% (v/v) glycerol, 30 µmol glucose-6-P, 4 gmol NADP+, 6 mg p-nitroblue tetrazolium, and 0.5 mg phenazine methosulfate.

Lactic dehydrogenase isoenzyme

LDH isoenzyme was determined according to Lehnert and Berlet (1979). After electrophoresis, the gel was incubated with combination of water, Tris, tetrazolium-blue, Phenazine methosulphate, Na-lactate and NAD to develop colour reaction for 20 min. In the colour reaction, NAD and lactate served as substrate. Phenazine-methosulphate is the primary electron acceptor and tetrazolium-blue is the final electron acceptor.

MDH isoenzyme

Staining solution was carried out by mixing 50 mN Tris-HCl (pH 8.5), nicotinamide adenine dinucliotide (NAD), maleic acid (after neutralized with NaOH), nitro blue tetrazolium chloride (NBT) and phenozine methosulphate (PMS) (Atzpodienj et al., 1968).

Isoenzyme patterns were recorded on the basis of number and the rate of flow (Rf) of the bands. The Rf value is the mobility of each isoenzyme band that travelled from the origin divided by the distance travelled by the front tracking dye. The presence or absence of a certain isoenzymatic band was considered as a differentiating feature. Zymograms were drawn to scale and relative mobility values were calculated for each band.

Comet assay

The specimens were homogenized in phosphate buffered solution pH 7.5. About 6 µl of homogenate was suspended on 0.5% low melting agarose and put in between a layer of 0.6% normal-melting agarose and a top layer of 0.5% low melting agarose on fully frosted slides. Lysis was carried out of the slides followed by electrophoresis to allow unwinding of DNA. Electrophoresis was performed for 10 min at 300 mA and 1 V/cm. The slides were neutralized and stained with 20 mg/ml ethidium bromide. Each slide was analyzed using a Leitz Orthoplan (Wetzlar, Germany) epifluorescence microscope. One hundred cells were analyzed on each slide using the comet assay II automatic digital analysis system. Perspective tail length (mm) (DNA migration from the centre of the body of the nuclear core) was used to determine DNA damage (Olive and Banáth, 2006).

Statistical analysis

Data were presented as mean ± standard error. The statistical analysis was performed with multi-variant analysis of variance (MANOVA) using SPSS (version 13) software package for windows for comparing the multivariations between each specie and $P<0.05$ was considered statistically significant.

Results

Prostate biochemical changes

Table 1 illustrates the biochemical changes in prostate gland of diabetic and hypercholesterolemic groups. Comparing with the control, HSP-70, 8-HDG and activity of lactic dehydrogenase, malate dehydrogenase and glucose-6 phosphate dehydrogenase markedly increased in both testis and prostate gland in comparison with the control. However, there was a marked depletion of prostate contents of VEGF, adhesive molecules (V-CAM, I-CAM) as well as the enzyme activity of AST compared with the control.

Histological observations of prostate and thyroid gland

The prostate of control rat was composed of three distinct paired lobes designated as the ventral, dorsal, and lateral lobes. It is tubuloalveolar and consisted of epithelium-lined acini surrounded by a stromal matrix. The acini of the ventral lobes have a large degree of folding. The epithelium of these acini is basophilic and mostly columnar with occasional cuboidal cells that have basally located nuclei. A typical tubulo-alveolar organization, with well-developed acini and different degrees of epithelial folds and the presence of a wide acinar lumen was observed. Many papillary projections were observed in most acini. Sparse stroma was present between the prostatic acini. The acini are rather tightly packed (Fig. 1A). In experimental diabetic group, there was a marked atrophy and collapsing of the alveoli forming a mass of cells enclosed reddish inclusions suspected glandular secretion or internal haemorrhage. Some of the collapsed acini contained reddish inclusions. The epithelial lining the acini appeared atrophied with sign of hyperplasia (Fig. 1B). In experimental hypercholesterolemic rats, the acini attained considerable shrinkage with presence of eosinous secretion within the acini lumina. The stoma of the acini exhibited reddish deposits manifested internal haemorrhage. The lining epithelial cells appeared atrophied to almost squamous or short cuboidal epithelial cells (Fig. 1 C&D). Diabetic and hypercholesterolemic groups possessed massive atrophied and collapsed of the acini with almost vanished lamina. Marked atrophy of the tubules with focal stratification of their lining cells was also observed. Internal haemorrhagic spots were clearly observed in stroma of the acini (Fig.1 E & F).

Concerning thyroid gland, the control rats showed varying size lines mainly in cubical follicular cells with rounded vesicular nuclei and few parafollicular C cells. The lumen was enclosed by eosinophilic colloid material. Interfollicular cells and blood capillaries occupied the space between thyroid follicles. The follicles were separated by thin collagen fibers (Fig. 2A). In experimentally diabetic and hypercholesterolemic rats, there was apparent congestion of the follicle with marked reduction of their luminal size. Some follicles showed vacuolated colloid while others were devoid of colloid. Few numbers of follicles possessed exfoliation of cells within their lumina. The interfollicular tissue possessed either focal interstitial hemorrhage or congestion of blood vessels (Fig. 2B). Experimental diabetic and hypercholesterolemic groups exhibited massive distortion of the follicles with almost missing of some luminal compartment and reduction of their thyroglobulin. Vacuolated luminal colloid and intertubular hemorrhage were also detected (Fig. 2 C&D).

Isoenzyme electrophoresis

Figure 3 illustrated the electrophoresis of prostate isoenzyme AST, EST, G6PD, LD and MDH. AST expressed two isoenzyme fractions. Hypercholesterolemia expressed three isoenzyme fractions

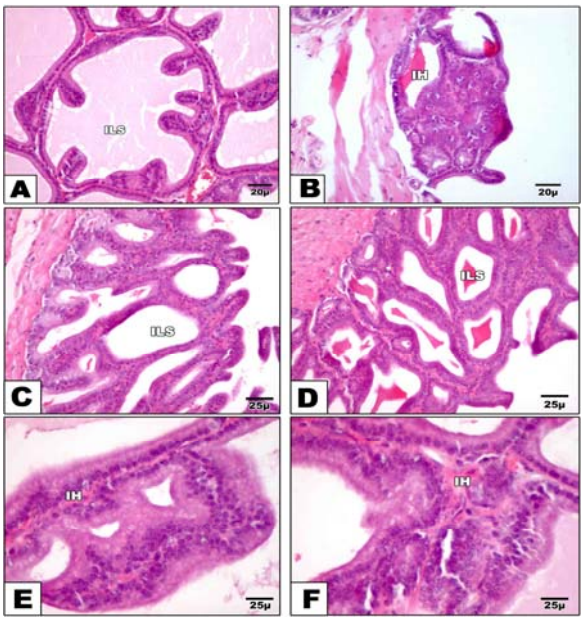

Fig. 1 (A-D): Photomicrograph of histological sections of prostate gland. A: Control showing normal folded acini. B: Diabetic showing congestion and missing of acini lumina and formation of prostate mass. C &D: Hypercholesterolemic showing massive congestion of acini and presence of eosinophilic inclusions in the lumina, collapsed acini with almost missing their lumina. (Abbreviation; IH : Internal haemorrhage, ILS : Interluminal space) HX-E

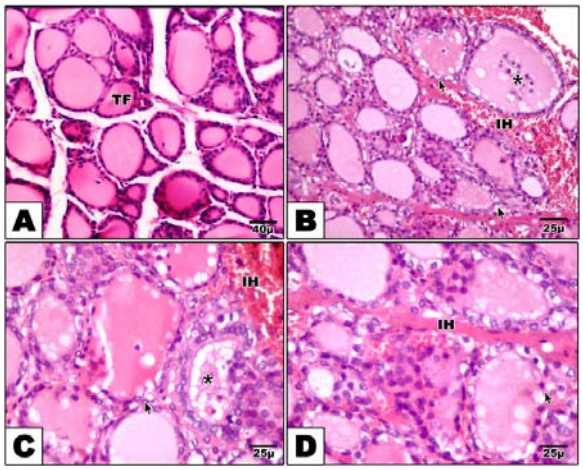

Fig. 2 (A-D): Photomicrograph of histological sections of thyroid gland. A: Control showing normal pattern of thyroid follicle and colloid. B: Diabetic showing reduced follicle and interfollicular haemorrhage. C: Hypercholesterolemic showing reduced follicle and interfollicular haemorrhage. D: Diabetic and hyper-cholesterolemic showing massive reduction of thyroid follicles and reduction of colloid. *Note vacuolation of colloid manifesting depletion. (Abbreviations; IH, internal hemorrhage; TF, thyroid follicle) HX-E.

comparing with the other experimental groups. There is a slight increase in densities of band comparing with the control. EST revealed the expression of five isoenzyme fractions. The density of isoenzyme fraction III is markedly increased in hypercholesterolemic group compared with the other experimental groups. In control, the isoenzyme fraction V is denser and almost missing in the experimental diabetic and or hypercholesterolemic groups. G6PD expressed four isoenzyme fractions. The enzyme showed marked increase of the isoenzyme fractions in experimental group. Over expression of extra band V was detected in diabetic group. Concerning LDH, three isoenzyme fractions were observed. Hypercholesterolemic group exhibited increased intensities of the isoenzyme fractions compared with the other experimental groups. Malate dehydrogenase expressed seven isoenzyme fractions. Treatment varied in expression of malate dehydrogenase. Diabetes expressed missing of the isoenzyme fractions I, II, V & VI. Hypercholesterolemia expressed missing of the isoenzymes 5, 6 & 7. However, combined treatment revealed missing of expression of isoenzyme fractions I &II and fading of isoenzyme fraction V. There is a considerable increase in the intensities of the isoenzyme fractions in prostate of hypercholesterolemic compared with diabetes and or hypercholesterolemia which seemed to be slightly changed.

Comet DNA damage

DNA of prostate tissue was damaged in diabetic and hypercholesterolemic gland manifested by detached and increased tail length compared with normal pattern structure of control (Fig. 4).

Discussion

The present findings revealed marked congestion and apparent missing of the lumin of prostate acini in diabetic and or hypercholesterolemic. Damage of epithelial lining cells and increase of internal haemorrhagic foci were also detected. Similar prostatic disease was reported in patients with atherosclerotic emboli vascular disease (Izquierdo-García et al., 2005). Azab (2013) reported increased daytime frequency and nocturia in hyperlipidemic patients. Diabetic patients were found to show enlargement of prostate with sign of clinical benign prostatic hyperplasia (Berger et al., 2006). The observed prostate damage was parallel with massive deterioration of thyroid gland manifested by degeneration of follicular epithelium, internal haemorrhage and apparent reduction of thyroglobulin.

Diabetic rats were found to possess marked depletion of thyroid hormones tT4, fT4 and tT3, as well as adenyl cyclase and thyroid stimulating hormone (Moyseyuk et al., 2014). These coincide with data

Table 1: Biochemical changes in prostate of diabetic and or hypercholesterolemic male rats

	Prostate					
	C	D	H	H+D	*F-test*	P ≤ 0.05
VEGF (Pg/mg)	25.7±1.3	22.6±1.2**	24.1±1.4*	21.1±1.4*	2.884	NS
HSP-70 (ng/mg)	30.6±1.2	40.6±1.6**	35.2±1.2*	45.1±0.9**	14.87	S
HDG (ng/mg)	11.7±1.1	20.3±1.3**	17.9±1.2**	25.4±0.9**	54.03	S
V-CAM (Ng/mg)	4.8±0.5	4.6±0.4*	4.0±0.1*	4.3±0.7*	1.029	NS
I-CAM (ng/mg)	3.5±.3	3.3±0.1*	3.4±0.1*	3.1±0.1*	1.331	NS
MDH (U/l)	48.1±1.4	52.6±1.1*	45.2±1.3*	59.4±1.3**	6.982	S
LDH (U/l)	67.4±1.7	85.3±1.1**	78.8±1.7**	79.2±1.3*	24.69	S
AST (U/l)	95.6±1.6	77.2±0.8**	88.1±1.0**	62.8±1.4**	122.4	S
G6PHD (Nmol/dl)	35.2±0.8	42.4±1.1**	43.1±1.6**	43.7±0.7**	11.38	S

Each result represent M±SE (n=5); C, control; D, diabetes; H, hypercholesterolemia; H+D, hypercholesterolemia and diabetes; VEGF,vascular endothelial growth factor; 8-HDG, 8- hydroxy guanosine ;I-CAM, intrercellular adhesion molecule; V-CAM,vascular cell adhesion molecule; MDH, maleic dehydrogenase;LDH, lactic dehydrogenase; AST, aspartate aminotransferase; G6-PDH, glucose-6-phosphate dehydrogenase.* Non-significant at P < 0.05, ** Significant at P<0.05

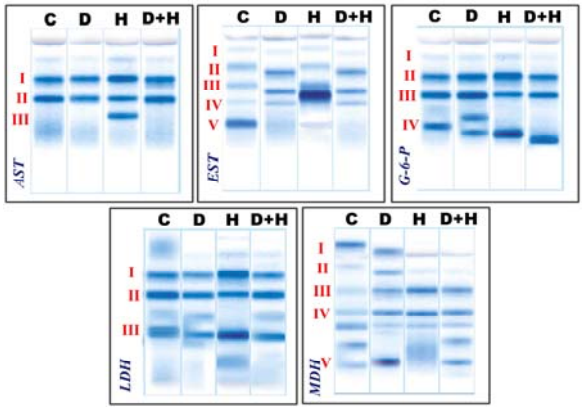

Fig. 3: Isoenzyme electrophoresis of AST, EST, G6PD, LDH & MDH of prostate gland of diabetic and or hypercholesterolemia. C: control, D: diabetes, H: hypercholesterolemia.

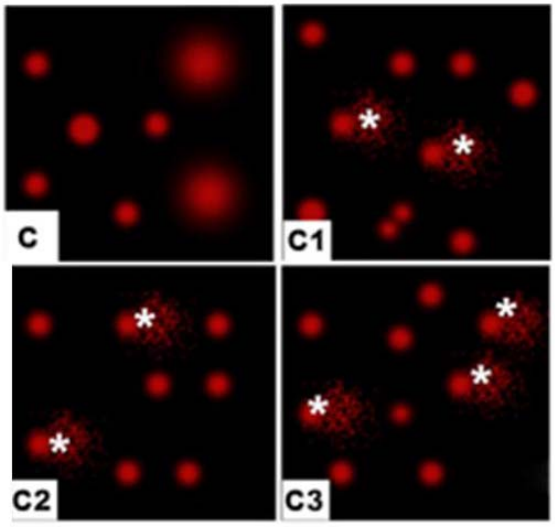

Fig. 4 (C-C3): Comet assay of prostate gland. C: Control, C1: Diabetic, C2: Hypercholesterolemia, C3: Diabetic and hypercholesterolemia. *Note detached prostate cells manifesting apoptosis

Table 2: Comet assay of prostate gland

	Prostate	
	Tail length (µm)	DNA%
C	1.85±0.10	1.92±0.19
D	7.13±0.69	5.45±0.55
H	4.43±0.35	4.43±0.28
HD	10.93±0.68	10.01±0.94
F-test	55.546	117.646
P≤0.05	S	S

Each result represent M±SE (n=5); C, control; D, diabetes; H, hypercholesterolemia; H+D, hypercholesterolemia and diabetes, ** Significant at P<0.05

reported in humans (Gopal et al., 2014; Kalra, 2014; Tereshchenko and Suslina, 2014; Araujo et al., 2015) who reported increased incidence of thyroid dysfunction in patients with type 1 diabetes mellitus. Rabbits with experimental hypothyroidism were found to cause depletion of endothelium derived relaxant factor thereby causing very feeble contraction of the cavernosum muscle and decrease erectyl function (Amadi et al., 2006). From the findings of histopathological changes, prostate revealed apparent damage which was confirmed by comet assay and heat shock protein. Similar findings of DNA were reported by Shi et al. (2015) in prostate cancer. Heat shock factor 1 (HSF1) was found to be associated with tissue-specific tumorigenesis in a number of mouse models, and has been used a as prognostic marker of cancer types, including breast and prostatic cancer (Li et al., 2014). Reeves et al. (2013) mentioned that there were reduced adhesive interactions between prostate cancer and bone marrow endothelial cells. Similar findings of decreased VEGF was reported by Joseph et al. (1997) who found marked depletion of VEGF in prostate tissue and an increase in prostate fluids and secretions.

It is well known that AST catalyzes the reversible transfer of α-amino group between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism which is important for

preserving the prostate function. Also Esterase isoenzymes are over expressed in cancer cells (McGoldrick et al., 2014). Yamazaki et al. (1998) examined the esterase activity profiles of various human and animal cancer tumours and reported increased activity in cancer cells. The observed increased expression of esterases especially in hypercholesterolemia reflects the damage of prostate. Normal and cancer cells were found to differ from one another with respect to mitochondrial LDH protein level and activity. Both enzymes LDH and malate dehydrogenase were more highly expressed and being of higher activity in prostate cells (De Bari et al., 2010 & 2013; Liu et al., 2013). From the present findings both quantitative assessment and qualitative G6PD expression in prostate, there was a detected increase of the enzyme activity which was parallel with the over-expression of the isoenzymes especially in diabetes. Similar findings were reported by Zampella et al. (1982) who reported four folds increase of the G6PD in prostate cancer.

It was concluded that diabetes and or hypercholesterolemia led to marked alteration of thyroid gland and prostate gland as assessed by biochemical markers, comet assay and isoenzyme electrophoresis.

References

Alba FM, Wang R (2012) Can erectile function be predicted after prostate câncer treatment? Asian J Androl 14: 134-135.

Amadi K, Sabo MA, Sagay AS (2006) Thyroid hormone: the modulator of erectile function in the rabbit. Niger J Physiol Sci 21: 83-89.

Andrews AT (1986) Electrophoresis: theory, techniques and biochemical and clinical applications. 2nd ed. New York: Oxford University Press.

Araujo DB, Barone B, Melleti NF, Dantas JR, Oliveira MM, Zajdenverg L, Tortora RP, Vaisman M, Milech A, Oliveira JE, Rodacki M (2015) Thyroid disorders are common in first-degree relatives of individuals with type 1 diabetes mellitus. Arch Endocrinol Metab 59: 112-115.

Atzpodienj W, Gancedow M, Duntze W, Holzer H (1968) Isoenzymes of malate dehydrogenase in Saccharomyces cerevisiae. Eur J Biochem 7: 58-62.

Azab S (2013) The impact of atherosclerosis on lower urinary tract function. Aging Male 16: 108-11.

Beebee T, Carty D (1983) A study of lactate dehydrogenase levels and turnover rates during postnatal development in the rat. Biochim Biophys Acta 757: 209.

Berger AP, Bartsch G, Deibl M, Alber H, Pachinger O, Fritsche G, Rantner B, Fraedrich G, Pallwein L, Aigner F, Horninger W, Frauscher F (2006) Atherosclerosis as a risk factor for benign prostatic hyperplasia. BJU Int 98: 1038-42.

Black MM, Gawkrodger DJ, Seymour CA, Weismann K (1998) Metabolic and nutritional disorders. In: Champion RH, Burton JL, Burns DA, Breathnach SM, editors. Textbook of Dermatology. 6th edn. Oxford: Blackwell-Science; pp: 2577–677.

Cappelleri JC, Rosen RC (2005) The sexual health inventory for men (shim): a 5-year review of research and clinical experience. Int J Impot Res 17: 307–319.

Casey RG, Corcoran NM, Goldenberg SL (2012) Quality of life issues in men undergoing androgen deprivation therapy: a review. Asian J Androl 14: 226-31.

De Bari L, Chieppa G, Marra E, Passarella S (2010) L-lactate metabolism can occur in normal and cancer prostate cells via the novel mitochondrial L-lactate dehydrogenase. Int J Oncol 37: 1607-1620.

de Bari L, Moro L, Passarella S (2013) Prostate cancer cells metabolize d-lactate inside mitochondria via a D-lactate dehydrogenase which is more active and highly expressed than in normal cells. FEBS Lett 587: 467-73.

Donohue T, Mahowald T, Adams D, Barker K (1981) Glucose-6-Phosphate dehydrogenase. partial characterization of the rat liver and uterine enzymes. Biochim Biophys Acta 658: 358.

Dupont C, Ralliard-Rousseau D, Tarrade A, Faure C, Dahirel M, Sion B, Brugnon F, Levy R, Chavatte-Palmer P (2014) Impact of maternal hyperlipidic hypercholesterolaemic diet on male reproductive organs and testosterone concentration in rabbits. J Dev Orig Health Dis 5: 183-8.

El-Sayyad HI, Al-Haggar MS, El-Ghawet HA, Bakr IH (2012a) Cardiomyopathy and angiogenesis defects of Wistar rat fetuses of diabetic and hypercholesterolemic mothers. Nutrition 28: e33-43.

El-Sayyad HI, Khalifa SA, Fouda YA, Yonis AS (2012b) Effects of diabetes and or hypercholesterolemia on rat foetuses skin development. Nutrition 28: 698-706.

El-Sayyad HIH, El-Ghawet HA, Al-Haggar MS, Iman H. Bakr (2015b) Impairment of bone growth of wistar rat fetuses of diabetic and hypercholesterolemic mothers. Egyptian J Basic Appl Sci 2: 1–12.

El-Sayyad HIH, El-Mansi AA, Bakr IH (2015a) Hypercholesterolemia induced ocular disorder: Ameliorating role of phytotherapy. Nutrition. DOI: http://dx.doi.org/10.1016/j.nut.2015.05.013.

El-Sayyad HIH, El-Sherbiny M, Sobh MA, Abou-El-Naga AM, Ibrahim MAN, Mousa SA (2011) Protective effects of Morus alba leaves extract on ocular functions of pups from diabetic and

hypercholesterolemic mother rats. Int J Biol Sci 7: 715-728.

El-Sayyad, HIH, Al-Haggar, MMS, El-Ghawet, HA, Bakr, IHM (2014) Effect of maternal diabetes and hypercholesterolemia on fetal liver of albino Wistar rats. Nutrition 30: 326–336.

El-Sayyad, HIH (2015) Cholesterol overload impairing cerebellar function: the promise of natural products. Nutrition 31: 621-630.

Enkhmaa B, Shiwaku K, Anuurad E, Nogi A, Kitajima K, Yamasaki M, et al. (2005) Prevalence of the metabolic syndrome using the Third Report of the National Cholesterol Educational Program Expert Panel on Detection, Evaluation, and Treatment of HighBlood Cholesterol inAdults (ATPIII) and themodified ATPIII definitions for Japanese and Mongolians. Clin Chim Acta 352:105–113.

Gaal O, Medgyesi GA, Vereczkey L (1980) Electrophoresis in the Separation of Biological Macromolecules. John Wiley & Sons, Chichester, pp: 83-87.

Gopal KV, Rao GR, Kumar YH (2014). Increased prevalence of thyroid dysfunction and diabetes mellitus in Indian vitiligo patients: A case-control study. Indian Dermatol Online J 5: 456-60.

Itoh H, Srere PA (1970) A new assay for glutamate-oxaloacetate transaminase. Anal Biochem 35: 405-410.

Izquierdo-García FM, Reimunde-Seoane E, González Medina A, García Díez F, Suarez-Vilela D, Ribas-Ariño T, Roa-Luzuriaga M (2005) Cholesterol embolism of the upper urinary tract as a clinical feature of atherosclerotic embolic vascular disease. Report of three cases, one of them simulating neoplasia. Arch Esp Urol 58: 867-872.

Joseph IBJK, Nelson JB, Denmeade SR and Isaacs JT (1997) Androgens regulate vascular endothelial growth factor content in normal and malignant prostatic tissue. Clin Can Res 2507: 2507-2511.

Kalra S (2014) Thyroid disorders and diabetes. J Pak Med Assoc 64: 966-968.

Krycer JR, Brown AJ (2013) Cholesterol accumulation in prostate cancer: a classic observation from a modern perspective. Biochim Biophys Acta 1835(2):219-29.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Lowry OH, RosehmughNJ,Farr AL, Randall KJ (1951) Protein medsurelnent wltb the folin phenol reagent. J. Biol Chem. 193: 265-275.

Lehnert T, Berlet HH (1979) Selective inactivation of lactate dehydrogenase of rat tissues by sodium deoxycholate. Biochem J 177: 813–818.

Li S, Ma W, Fei T, Lou Q, Zhang Y, Cui X, Qin X, Zhang J, Liu G, Dong Z, Ma Y, Song Z, Hu Y (2014) Upregulation of heat shock factor 1 transcription activity is associated with hepatocellular carcinoma progression. Mol Med Rep 10: 2313-21.

Liu Q, Harvey CT, Geng H, Xue C, Chen V, Beer TM, Qian DZ (2013). Malate dehydrogenase 2 confers docetaxel resistance via regulations of JNK signaling and oxidative metabolism. Prostate 73: 1028-1037.

López-Soler N, Cervera A, Moores GD, Martínez-Pardo R, Garcerá MD (2008) Esterase isoenzymes and insecticide resistance in Frankliniella occidentalis populations from the south-east region of Spain. Pest Manag Sci 64: 1258-1266.

McGarry JD (1992) What if Minkowski had been ageusic? An alternative angle on diabetes. Science 258: 766–70.

McGoldrick CA, Jiang YL, Paromov V, Brannon M, Krishnan K, Stone WL. Identification of oxidized protein hydrolase as a potential prodrug target in prostate cancer. BMC Cancer 14: 77-90.

Moyseyuk IV, Derkach KV, Shpakov AO (2014) The functional activity of thyroid of male rats with acute and mild streptozotocin diabetes. Zh Evol Biokhim Fiziol 50: 275-84.

Olive PL, Banáth JP (2006) The comet assay: a method to measure DNA damage in individual cells. Nat Protoc 1: 23-29.

Pelucchi C, Serraino D, Negri E, Montella M, Dellanoce C, Talamini R, La Vecchia C (2011) The metabolic syndrome and risk of prostate cancer in Italy. Ann Epidemiol 21: 835-841.

Peppa M, Uribarri J, Vlassara H (2003) Glucose, advanced glycation end products, and diabetes complications: what is new and what works. Clin Diabetes 21: 186–187.

Povoski SP, McCullough PJ, Zhou W, Bell RH (1993) Induction of diabetes mellitus in Syrian golden hamsters using stored equilibrium solutions of streptozotocin Lab Anim Sci 43: 310–14.

Rabijewski M, Papierska L, Piątkiewicz P (2014) The prevalence of prediabetes in population of Polish men with late-onset hypogonadism. Aging Male 17: 141-146.

Reeves KJ, Hou J, Higham SE, Sun Z, Trzeciakowski JP, Meininger GA, Brown NJ (2013) Selective measurement and manipulation of adhesion forces between cancer cells and bone marrow endothelial cells using atomic force microscopy. Nanomedicine (Lond) 8: 921-34.

Shi W, Dong L, Bao JS (2015) Progress in the studies of prostate cancer related molecules. Zhonghua Nan Ke Xue 21: 357-62.

Tereshchenko IV, Suslina AA (2014) Thyroid dysfunction in patients with type 2 diabetes mellitus. Ter Arkh 86: 119-23.

Wolfe RG, Neilands TB (1956) Some molecular and kinetic properties of heart malic dehydrogenase. J Biol Chem 221: 61-69.

Xu Q, Willeit J, Marosi M, Kleindienst R, Oberhollenzer F, Kiechl S, Stulnig T, Luef G, Wick G (1993) Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis. Lancet 341: 255-259.

Yamazaki Y, Furukawa F, Nishikawa A, Takahashi M, Oka S (1998) Histochemicaldetermination of stereoselectivity of esterases in normal pancreas and pancreatic tubular adenocarcinoma of hamsters. Biotech Histochem 73: 23-31.

Zampella EJ, Bradley EL Jr, Pretlow TG (1982) Glucose-6-phosphate dehydrogenase: a possible clinical indicator for prostatic carcinoma. Cancer 49: 384-387.