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Molecular Aspects of Diabetic Heart

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KEYWORDS

Diabetes mellitus (DM)**,** abnormalities, myocardium, total cholesterol (TC),Low density and high density lipoproteins (LDL & HDL) and triglyceride (TG).

ABSTRACT:

Diabetes mellitus (DM) is characterised by hyperglycemia due to disturbances in the metabolism of carbohydrate, fat and protein because of abnormalities in the availability of insulin or insulin-action. The secondary complications seen in diabetic patients are found to involve alterations in vascular basement membrane composition as well as accumulation of glucose derived reaction products due to over utilization of glucose in insulin independent tissue (Sprio, 1976). The proteins of the myocardium are the most important part of the socalled myofilaments whose unit is the sarcomere (Solaro and Rarick 1998). The concentration of serum lipids – total cholesterol (TC), low density and high density lipoproteins (LDL & HDL) and triglyceride (TG) is another important index of overall metabolic control in diabetic patients and should be measured at diagnosis and regularly thereafter (Frier and Fisher, 2006).

Introduction

Diabetes mellitus (DM) is characterised by hyperglycemia due to disturbances in the metabolism of carbohydrate, fat and protein because of abnormalities in the availability of insulin or insulin-action. Even though diabetes mellitus is an endocrine disease in origin, its major manifestations are those of a metabolic disease. The characteristics symptoms are excessive thirst, polyuria and otherwise unexplained weight loss. Diabetes also brings about the progression of secondary complications through the thickening of basement membrane (WHO, 1994). The most dominant feature of the metabolism in diabetes mellitus is an abnormally high concentration of blood glucose. This can be either due to an abnormally high rate of glucose production or of impaired glucose utilization. It is now accepted that the high blood glucose level is the result of combination of both these processes.

The secondary complications seen in diabetic patients are found to involve alterations in vascular basement membrane composition as well as accumulation of glucose derived reaction products due to over utilization of glucose in insulin independent tissue (Sprio, 1976). Various authors have shown that hyperglycemia leads to an increase in serum glycated proteins (Mani *et.al.,* 1987), along with alterations in other atherogenic risk factors. Further, disturbances in mineral metabolism are also noticed (Walter *et.al.,* 1991), and it is not known whether differences in trace element status are a consequence to the expression of the disease.

EPIDEMIOLOGY:

The Diabetes is a disorder that occurs worldwide, there are an estimated 246 million people with diabetes, and this number is set to reach 380 million by 2025. Diabetes was traditionally considered to be a disorder affecting the affluent and the elderly. India holds the unenviable position

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of being the world leader with 40 million diabetics in 2007. This number is set to reach 70 million by 2025. In more advanced stage of the disease when 90% of the kidneys are damaged life threatening heart failure and uremic coma may develop.

Previous studies investigating the extent of ischaemic injury in diabetic myocardium have been evidenced that the diabetic heart is highly sensitive to such injury. The streptozotocin model which they used has been shown to reliably produce diabetes in rats with minimal systemic toxicity and is characterized by substantial increases in fasting blood glucose concentration.

Heart failure:

The condition defined as heart failure or cardiac insufficiency refers to a complex syndrome that can be eitherproduced by any major cardiac disorder over a more orless extended period of time or induced acutely whenthere is a sudden or rapid destruction of cardiac cells (Dallavolta, 1993). In both instances, the normal mechanisms governingheart activity are lost, initially because the need for replacementof normal cardiac cells with new elements creates a new simpler genetic program which modifiesthe formation of the normal cellular and interstitial elements,involving a fetal type of myosin and thick new collagen.

A shortened lifespan results, as these new cellularelements are not able to sustain normal physiological activitiesand die either of spontaneous apoptosis or fromincreased necrosis. A progressive remodelingof theheart ensues, because the heart is unable to meet the requirementsof the body, thereby inducing external acceleratedneural, hormonal, and cellular interventions (Dallavolta, 1990). These interventions produce a progressive reduction ofthe normal, physiological, aspects of circulation, a naturalexample of the application of the second principle of thermodynamics,the law of Clausius, which states that an increasedrate of progression evolves toward maximalchaos. In clinical terms, this means the complete inabilityto maintain equilibrium, eventually leading to the death ofthe patient.

The new genetic program (Thaik *et al.,* 1995) produces less than optimal cardiac cells and altered transport of Na, K and Ca ions through their channels, reduced contractility,altered action of the determinants of the cardiacpump, accelerated cellular death, the need for externalneurohormonal intervention (Leimbach *et.al*., 1986; Vikstrom and Leinwand 1996).Redistribution of theblood in the different parts of the body, and progressiontoward a more and more accelerated circulatory instability.This condition is irreversible (Sandoshima and Izuma 1997). without therapeutic interventionor, better, prevention of the development of heart failure, when a cardiac disorder capable of inducing heart failure was present.

Proteins of the myocardium:

The proteins of the myocardium (**Figure 1**) are the most important part of the socalled myofilaments whose unit is the sarcomere(Solaro and Rarick 1998). Thesarcomere is composed basically in its central portion of thickfilaments, the myosin, while the lateral regions are formed bythin filaments, the actin.The spatial shape of the sarcomere is hexagonal (Moss *et al.,* 2004). The proteins of any sarcomere are usually dividedinto three main groups, according to their functions:

The ***contractile proteins,*** which produce the apparentshortening of the sarcomere that in reality is the slippingof the heads of the thick over the thin filaments.The bridge-forming union of myosin heavy chain headswith the thin filaments of actin forms the ultrastructuralbasis of cardiac contraction.The ***modulating proteins***,troponins, tropomyosin andtropomerosin, whose detachment from the actin ismade possible by the arrival of Ca2+ and combinationwith it. The transient arrival of small amounts of Ca2+ intothe sarcolemma blocks the inhibition of the union ofactin and myosin.The ***anchoring proteins*** whose tethering prevents unnecessary displacement of the cardiac cells during the slippage of myosin on actin.

DIAGNOSIS

Diagnosis of diabetes can be based on the presence of suggestive symptoms together with lab results that support the specific diagnosis.IDDM is easy to diagnose based on clinical symptoms and lab values. The

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identification of certain auto antibodies is particularly helpful in diagnosing IDDM. The onset of IDDM is almost always rapid and dramatic. Rarely, onset can be gradual and result in a diagnostic dilemma in which it is difficult to distinguish from NIDDM, particularly in an individual who is age 35-50 and not obese. Testing for auto antibodies in such individuals can help distinguish the two diseases.

ANTIDIABETIC ACTIVITY

Blood Glucose: Laboratory glucose testing in blood relies upon enzymatic reaction (glucose oxidase) and is cheap, usually automated and highly reliable. However, variation in blood glucose depends on whether the patient has eaten recently, so it is important to consider the circumstances in which the blood sample was taken (Frier and Fisher, 2006).

Blood glucose values are normally maintained in a very narrow range, usually 70-120mg/dL. The diagnosis of diabetes is established by noting elevation of blood glucose by any one of three criteria;

1. A random glucose >200mg/dL with classical signs and symptoms.
2. A fasting glucose >126mg/dL on more than one occasion.
3. An abnormal oral glucose tolerance test (OGTT), in which the glucose is >200mg/dL 2hrs after a standard carbohydrate load.

Levels of blood glucose proceed along a continuum. Individuals with fasting glucose <110mg/dL or <140mg/dL following an OGTT and are considered to be euglycemic. However, those with fasting glucose >100 but <126 or OGTT values >140 but <200 are considered to have impaired glucose tolerance (IGT). Individual with IGT have a significant risk of progressing to overt diabetes over time, with up to 5%-10% advancing to diabetes mellitus (DM) per year.

Blood Lipids

The concentration of serum lipids – total cholesterol (TC), low density and high density lipoproteins (LDL & HDL) and triglyceride (TG) is another important index of overall metabolic control in diabetic patients and should be measured at diagnosis and regularly thereafter (Frier and Fisher, 2006).

Experimental design:

The Wistar albino rats in the weight range of 110-120g were chosen for this study. The animals were divided into three group. Each group contains four animals. Animals were fed and libitum with gold mohur rat feed from Hindustan lever limited, Bangalore, India. The College ethical committee has cleared the animal experiments carried out in the project.

Group I: These animals were considered as normal control and animals were injected with normal saline in the route of intraperitonial.

Group II: Animals were injected with streptozotocin (50mg/kg body weight) in the route ofintraperitonial to induce diabetes.

Group III: Animals were injected with 182kDa (50µg/kg body weight) through tail vein to produce hypertrophy.

Induction of diabetes:

Intraperitonial injection of strpetozotocin at a dose of 50mg/kg body weight and control animals was given with normal saline for consecutive five days.

Preparation of serum:

After induction of diabetes, at different day point serial bleeding of rat was done by retro orbital puncture following the method of Mariappan et al.,1994. The blood was collected by the capillary action transferred to serology tube and kept at room temperature for 15 minutes. The Serum sample was used for protein estimation by Lowry's method (Lowry et al.,1951).

SDS Polyacrylamide Gel electrophoresis of proteins (15% gel)

Almost all analytical electrophoresis of proteins are carried out under conditions that ensure dissociation of the proteins into their peptide subunits and that minimize aggregation. The purified proteins were resolved in SDS-PAGE by discontunous buffer systems (Smith, 1994).

After the separating gel gets polymerized remove the over laid water carefully with a filter paper and insert an appropriate "comb" between the plates. The stacking gel mixture was mixed well and poured immediately (to the

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brim) over the separating gel. The stacking gel was allowed to polymerize. Add additional gel mixture if the gel retracts significantly and sample was prepared.

Preparation of Samples:

The samples were mixed with sample buffer and were boiled for 5 minutes at 100ºc, centrifuged at 3000 rpm for 5 min. the sample volume containing 50-100µg protein was loaded in the wells. Electrophoresis was carried out at a constant voltage of 100V across the gel. Run the gel towards anode, monitoring the electrophoresis with the movement of Bromophenol blue. At the end of the run switch off the power pack, detach the plates from the tank. Laid the gel and the plates flat on the table and lift up a corner of the upper glass plate and pull it smoothly away. After gel was taken carefully and bands was identified by stained and destained method.

Stainingand Destaining:

The gel was immersed in staining solution (0.25% coomassive brilliant blue R-250 in 100ml of 50% ethanol containing 7% (v/v) acetic acid) and was gently shaken through the process. The process was carried out for 4-6 hours. Later the gel was destained in the destaining solution (50% ethanol/methanol and 7% acetic acid) in order to remove the background, once the bands are clear the gel was stored in 7% acetic acid.

Purification of 182 kDa protein:

182kDa hypertrophic serum protein was purified using Blue sepharose column chromatography. DEAE-sepharose CL6B column chromatography and HPLC by the method of Mariappan *et al.,* (1994). Briefly total serum protein were chromatographed on a CM off-gel-blue column, precipitated by the addition of ammonium sulphate to 50% s saturation and dialysis against the buffer containing 50mM Tris-Hcl, pH8.0, 5mM $Mgcl_2$ and 0.1mM PMSF at 4ºC for 16 hours with four changes. Further the fraction was applied to a DEAE sepharose CL6B column and eluted with linear gradient of 0-500mM Nacl in 50mM tris-Hcl pH8.0 and 5mM $Mgcl_2$. The 75-125mM Nacl eluted fractions from DEAE-sepharose column containing the 182kDa protein were pooled and fractioned in BIORAD HPLC system using a gel filtration column (300mmx7.8mm). The fraction containing the 182kDa protein was collected and stored at -70ºC.

Histopthological examination:

Hearts were fixed in 10% formalin and sectioned along the axis through the atria and ventricle of experimental heart. The sections were stained with hematoxylin eosin.

ESTIMATION OF PROTEIN BY LOWRY'S METHOD (1951)

Procedure:

1. A test tube was taken, 1ml of 1N NaOH was transferred into a test tube. The solution was heated up to 100ºC
2. 10µl of protein sample (serum) was suspended into the above solution for 4 to 5 minutes
3. 5ml of reagent D (alkaline $CuSO_4$ mix properly and leave the mixture at room temperature for 10minutes) was added.
4. 0.5ml of folin ciocaltue reagent was added with immediate mixing
5. After 30 minutes then the absorbance was seen.
6. Standard curve was plotted. (Concentration Vs absorbance) standard curve obtained by taking 0,5,15,20,25µg of BSA protein from the above procedure.

Western Blot analysis of diabetic serum protein with antirat 182kDa protein antibody:

In order to identify the presence of 182kDa hypertrophic specific protein, 200µg of serum proteins from control, diabetic and aorta constricted rat (positive control) were resolved on 15% SDS-PAGE and transferred to nitrocellulose membrane(NC). The transferred proteins were allowed to react with antihypertropic 182kDa protein antibody and antirabbit IgG HRP conjugated antibody. The intensity of colour formation in peroxidase reactions was monitored.

Procedure:

- Sample was prepared and SDS-PAGE was run.
- Gel was placed in electroblot apparatus with nitrocellulose filter on anode side.

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- Transferred at 30V constant voltage for 1 hour to overnight at 4ºC, depending on the specific experiment.
- Transferred proteins were visualized by staining in ponceau S solution for 5 minutes, destained in water for 2 to 3 minutes and marked molecular weight markers with indelible ink, destain for 10 min.
- Filter was blocked with blocking buffer for 1 hour at room temperature.
- Primary antiobody(antihypertrophic 182 kDa protein antibody) was diluted in blocking buffer (1:100 ratio) and incubated with the filter for 1 hour at room temperature.
- The filter was washed four times in 200 ml PBS for 15 min each wash.
- Secondary antibody (HRPO anti Ig conjugate) was diluted in blocking buffer (1:100 ratio) and incubated with the filter for 1 hour at room temperature.
- The filter was washed four times in 200 ml PBS for 15 min each wash.
- DAB substrate solution was added and developed.
- The staining reaction was stoped by washing with water.
- The stained filter was photograph for permanent record.

Extraction of total RNA from animal tissue: (Chomczynski and Sachi 1987).

The ability to isolate intact RNA has important uses in cloning genes and in analyszing gene expression. The difficulty in RNA isolation is that most ribonucleases are very stable and active enzymes that require no cofactors to function. The first step in all RNA isolation protocol therefore involves lysing the cell in a chemical environment that results in denaturation of ribonucleases. RNA ZOL method is an easy answer to this complicated problem. The RNA ZOL method can be completed within 3 hours providing both high yield and purity of RNA preparation. RNA isolated with the use of RNA ZOL is non-degraded, free of DNA and protein and contains the whole spectrum of RNA, molecules, including small RNAs. The preparation is ready for dot blot hybridization, gel electrophoresis to detect specific mRNA by Northern blotting, poly A^+ selection by the Oligo dT-cellulose method, or may be used for S1 protection and other enzymatic assays.

Homogenization:

The tissue sample was homogenized with RNA ZOL (2ml per 100mg tissue) with few strokes in a glass Telflon homogenizer.

The use of guanidium to lyse cells was originally developed to allow purification of RNA from cells rich in endogenous ribonuclease. Guanidium denatures protein and thus inactivates any ribonucleases that were present.

RNA Extraction:

1. 0.2ml Chloroform was added per 2ml of homogenate

2. The samples were tightly closed and shaken vigorously for 15 seconds and let them stay on ice for 15 minutes.

3. The suspension was centrifuged at 12,000rpm (-4ºC) for 15 minutes.

RNA precipitation:

1. The aqueous phase was transferred to a fresh tube, added an equal volume of iso-propanol and stored the samples for 45 minutes at -20ºC.

2. Samples were centrifuged for 15 minutes at 12,000rpm (-4ºC). RNA precipitate (often invisible before centrifugation) formed a white pellet at the bottom of the tube.

RNA Transfer:

1. The gel was placed in 10X SSC for 1hour with gentle shaking
2. Whatman filter paper was used to make a wick on a stack of glass plates. The wrapped support was placed inside a large dish. The dish was filled with 10X SSC.
3. The gel was placed on the 3MM Paper and smooth out air bubbles.
4. A nylon membrane cut was placed to the size of the gel on top the gel.
5. 5 sheets were placed on to 3MM paper wet with water and 10X SSC sequentially on top of the membrane without bubbles.

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6. A stack of paper towel (6cm) was placed on top of the 3MM paper. Then a glass plate was placed over that and compressed with a 1kg weight.
7. The transfer was allowed to occur for overnight.
8. The filter was removed and rinsed it in 20X SSC for 5 min. and dried.
9. The filter was baked at 80ºC for 2hrs in vacuum oven. If it was nylon membrane, fixed the RNA by UV irradiation for 3min in a UV transillminator.

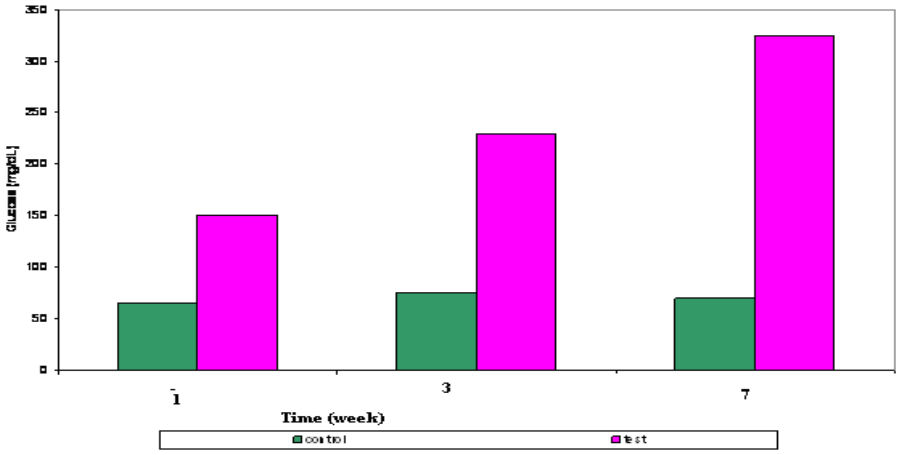

Figure 1. Level of blood glucose in control and diabetic rats

- **X-axis indicate, the blood sample was collected at different time duration (weeks).**
- **Y-axis indicate, the amount of glucose present in blood sample(mg/dL).**

To substantiate the role of 182 kDa protein in the development of cardiac hypertrophy, the effect of 182 kDa protein on the expression of muscle specific genes such as that of MLC_2 and β-MHC which were induced in pressure overloaded hypertrophy was investigated.

Purification of 182 kDa protein:

The 182 kDa hypertrophic serum protein was purified by using blue sepharose column chromatography. Figure 3.The purified protein was identified as 182 kDa protein by comparing with standard protein marker. The silver staining of protein (20 µg) contained in various DEAE fractions. It was indetified and separated by companing with standard protein marker it was shown in the figure 4. One of project gudie's previous research wrok has shown serum protein of 182 kDa, which is induced at a very early stage after the onset of hypertrophy in aorta-constriced experimental animals. A similar molecular wight protein has been indentified in the rat sera of diabetic induced experimental animal shown to be elevated in patients with various cardiac ailments such as ventricular septal defect, atrial septal defect, aortic regurgitation and aortic stenosis (Prabhkar and Rajamanickam, 1993).

Purification of CA2M protein by HPLC:

The CA2M protein was further purified by HPLC method. It was shown in the figure 5.

DEAC-sepharose fractions were identified and separated by comparing with standard protein marker.

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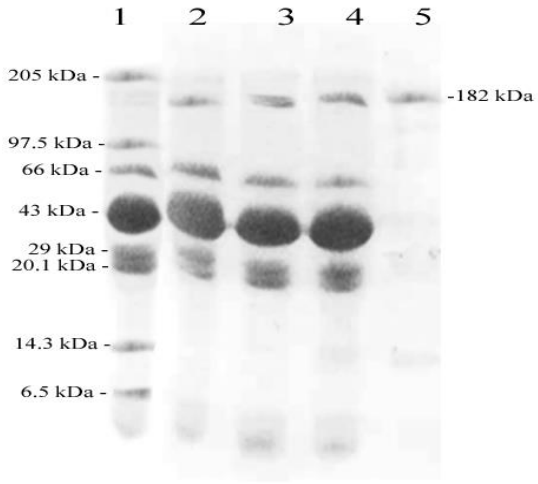
Figure 2.SEPARATION OF PROTEIN BY SDS -PAGE



Legends

I urchased protein marker

ug of protein

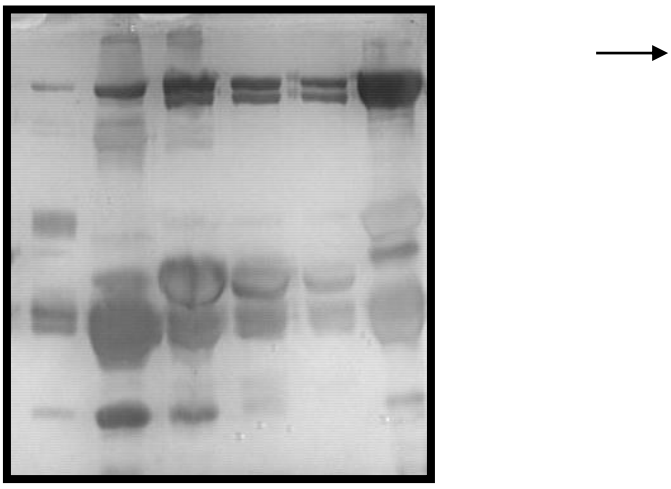
Lane 3: 5µg of protein

Lane 4: 7.5µg of protein

Lane 5: 10µg of protein

Figure 3.Silver staining of protein (20µg) contained various blue Sepharosefractions

1 2 3 4 5 6



Legends: - 1, 2, 3,4,5,6 various fractions clearly show the enrichment of CA_2M protein

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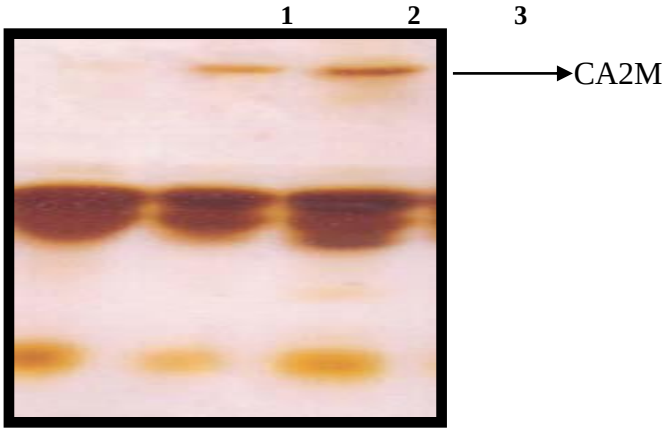
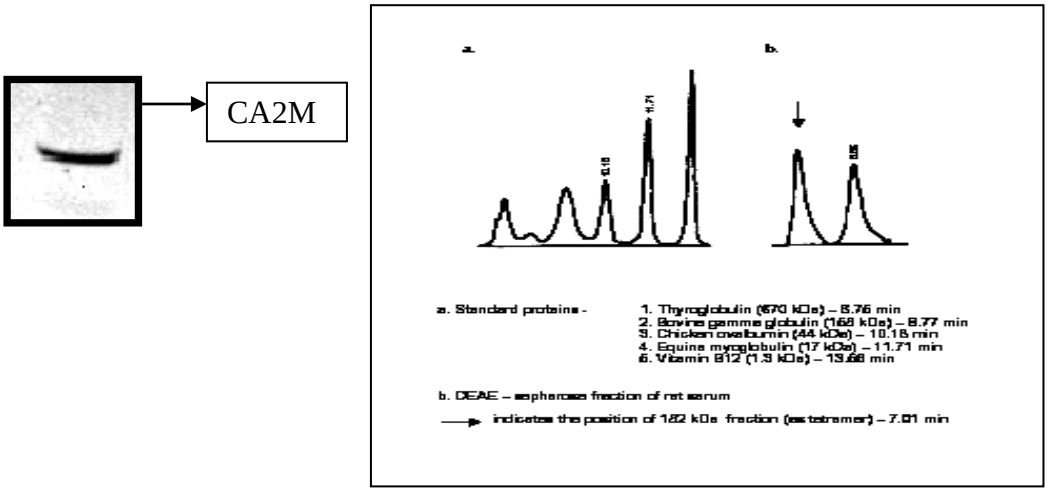
Figure 4. Silver staining of protein (20µg) contained various DEAE Fractions



Legends: - 1, 2, 3 the intensity of bands show the presence CA_2M Protein.

Figure 5.Purification of CA2M protein by HPLC



On comparison of heart weight / body weight rations of protein injected (IP) animals with that of controls are presented of Table 6. Biochemical comparison of 182 kDa serum protein from hypertrophy rats with α_2 macroglobulin.

The similarity of the molecular weigh of hypertrophy specific serum protein with that of α_2 macroglobulinand the appearance of both these proteins in the serum prompted a biochemical comparison of these two proteins and thereby a distinction between the two interms of their physiological

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functions. α_2 macroglobulin is a large glycoprotein found in the circulation of animals during acute inflammation. In order to check that the hypertrophy specific serum protein is also a glycoprotein, glycoprotein staining of both 182 kDa protein and α_2 macroglobulin were carried out and compared. The results showed that both these proteins belong to glycoprotein family.

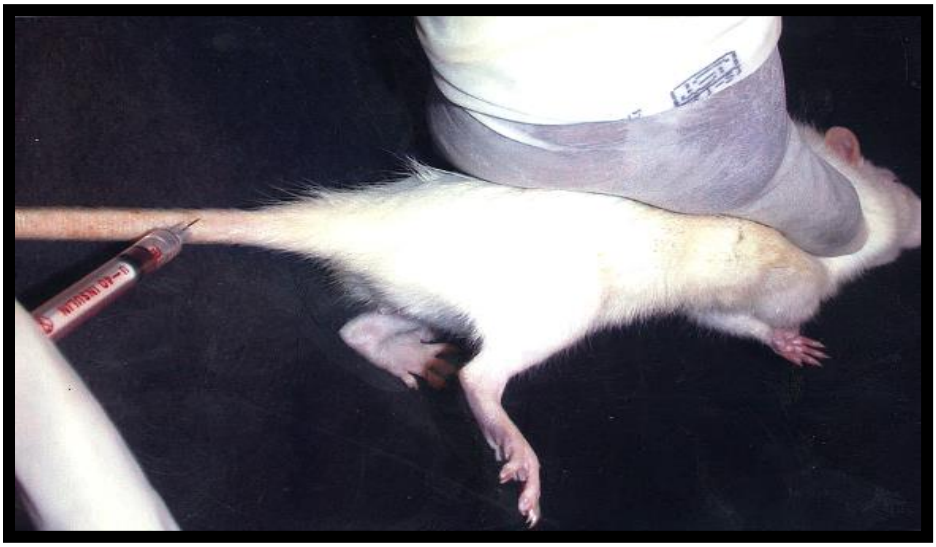
Table 6.Effect of CA2M on Diabetic Animals (Gravimetric Analysis)

	Heart wt.in mg	Body wt. in g	(Heart wt. /Body wt). x100
Normal	**124**	**33.3**	**372**
Diabetic **1st week** **5th week** **7th week**	**105** **95** **97**	**23** **21.7** **20**	**456** **436** **456**
Protein injected (25µg) 7th week	**117**	**26.1**	**420**

182kDa protein:

Direct injection of the purified 182kDa protein intravenously (through tail vein) into the normal animal led to development of cardiac hypertrophy. Multiple injections into the circulation of aorta constricted animals totally abolished the development of hypertrophy and down regulated the expression of β-MHC and MLC-2. The following figure 7. shown the purification of 182kDa protein. The purity of a protein is of prime importance in the 182kDa protein from the hypertrophied rat serum was standardized. The purified fraction was used to rise polyclonal antibody in rabbit for the detection of protein and assays.

Figure 7.Injection of 182 kDa protein in diabetic rat



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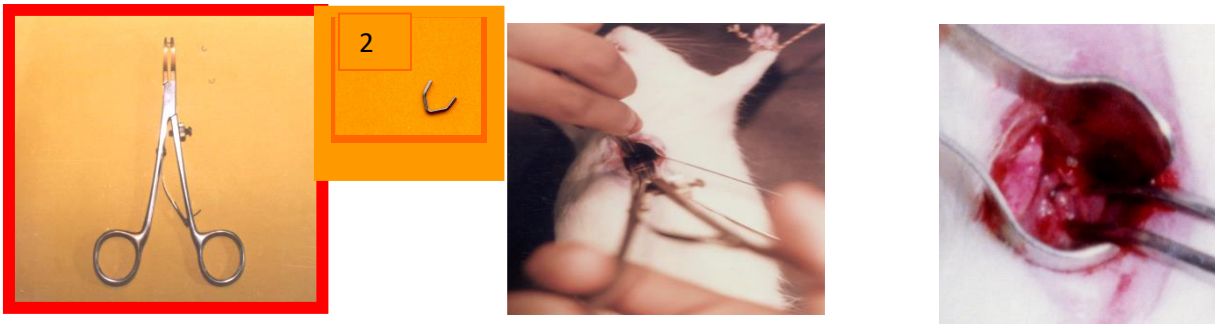
- DPC was injected to a set of 5 animals each 25µg intravenously (tail vein)
- A total of two injections were administered on 1st and 3rd day, after twelfth weeks of induction of Diabetes with Streptozotocin.

Figure 8. Induction of cardiac hypertrophy by aortic construction



1. Applicator & Surgical procedure for constricting
2. Tantalum Hemoclips the ascending aorta

Position of hemoclip on the aorta

Western Blot Analysis of CA2M in Diabetic Rats

The purified protein was also used to induce hypertrophy and elucidated the signal transduction pathways activity construction of cDNA library of a hypertrophied heart and RT-PCR approach were the strategies employed in cloning the full length cDNA of the 182kDa protein. As the protein expressed was secretary, it was detected in the serum and its presence was comforted by western blot analysis.

In order to elicit the differences between the two proteins, their immunological cross receptivity was checked by western blot analysis. The results showed that the α_2 macroglobulin a antibody reacted only with the α_2 macroglobulin not with the 182 kDa serum protein, where as the 182 kDa protein antibody reacted with both the 182 kDa and α_2 macroglobulin (western blot). However the immuno-cross reactivity was more intense with 182kDa serum protein then with α_2 macroglobulin. The lack of immunological cross reactivity of α_2 macroglobulin antibody with the 182 kDa serum protein implied that they may be two different proteins although they are similar in their molecular weight.

The present data provides evidence that the injected of streptozotocin can lead diabetic condition in animals, similarly hypertrophy inducing protein in the serum, have the molecular weight of 182 kDa, particular and appear in the blood stream.

These observations α_2 macroglobulin receptor, suggest a similar receptor mediated mechanism linking 182 kDa protein in the development of cardiac hypertrophy. There is a high probability that the cardiac specific 182 kDa protein may bind to some growth modulating facts and be targeted to cardiocytes where it can bind to some specific receptors as has been shown with α_2 macroglobulin and exert its grown stimulating property. An increase in the level of many growth factors, insulin like growth factor I and II have also been observed in diabetic rat heart due to pressure over load after aortic constricted.

Presence of CA2M protein in diabetic rats was identified by the western blot analysis was shown in figure 9.

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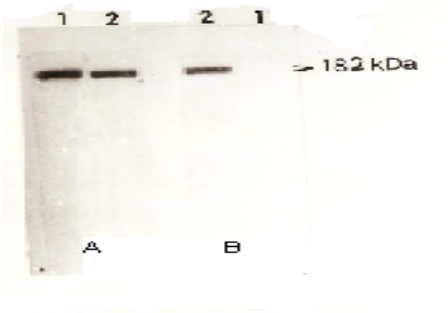
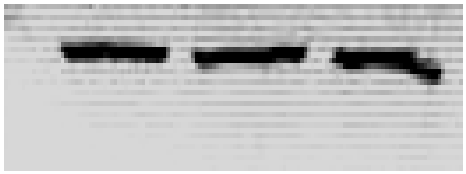
Figure 9. Western blotting analysis of CA_2M



Legends
Lane 1: 5µg of purified 182 kDa protein
Lane 2: 5µg α_2 macroglobulin
Western blot analysis of CA2M in Diabetic Rats

1 2 3

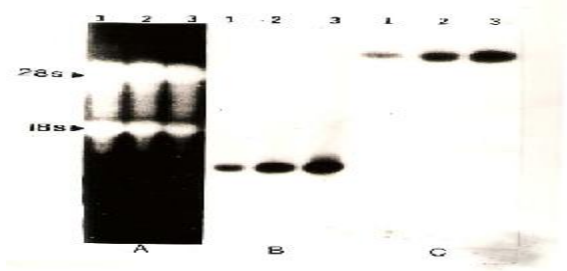


Lane 1, 2, 3 represents the presence of CA2M

Protein in Diabetic Rats from 1st to 7th Weeks

Presence of CA2M protein in diabetic rats was identified by the western blot analysis was shown in figure 9. The studies at present suggest that the 182kDa serum protein that appeared during early stage of development of diabetic rats was a glycoprotein which localized in the heart that showed immunological cress reactivity with α_2 macroglobulin and it was expressed in the heart as evinced by Northern blot analysis was shown figure 10.

Figure 10.Northern blot analysis



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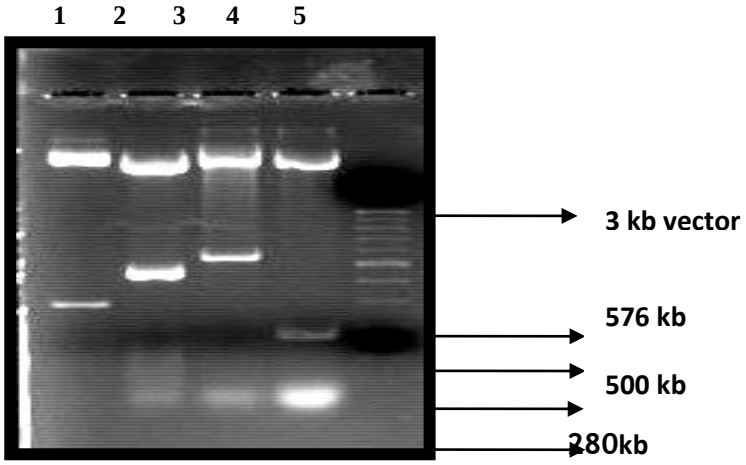
Legends:

Lene 1: **RNA of normal control animal**

Lene 2: **RNA of Protein injected animal**

Lene 3: **RNA of drug injected animal**

Probes used for Northern Blot Analysis (2% Agarose Gel)



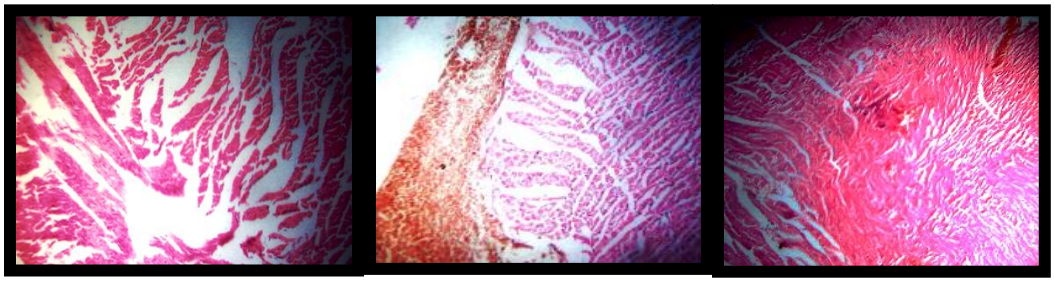
Lane 3: **ANF**

Lane 4: **β-MHC**

Lane 5: **100 bp Ladder**

Histopathological Examination

3mm cross sections of the heart tissues were embedded with glycomethocrylate and 4-7μm sections were cut and counterstained with hematoxylin and eosin as described previously. Photomicrosocpy showed cardiac necrosis that can be identified by their myofibrillan architecture, histological visualization of muscle specific gene expression in rat cardiac myocytes. It was shown in figure 11.



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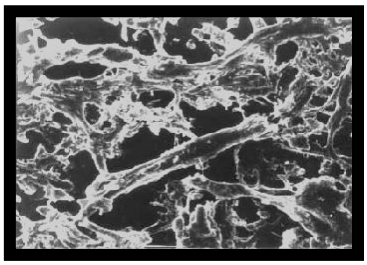
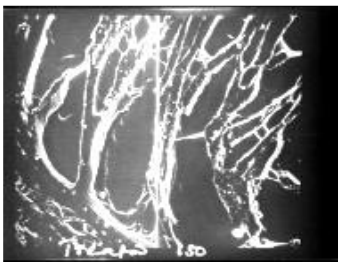
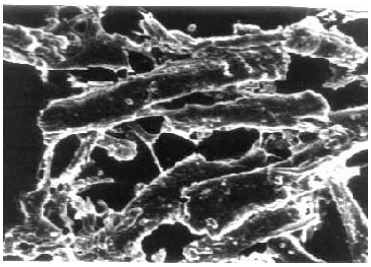
Normal **Diabetes** **Protein injected**







RESULT AND DISCUSSION

Diabetes was induced by injection of streptozotocin at dose of 50 mg/kg body weight for consecutive five days. This dose of streptozotocin has been demonstrated to produce moderate diabetes with impaired glucose level. The elevated blood glucose level in serum was estimated and shown in the figure 1.

- Blood glucose level was not increased in control
- In test animals increased glucose level was observed, and these diabetic animals did not gain as much weight over 7 weeks.

Diabetic complications:

Streptozotocin induced diabetes mellitus, caused by destruction of pancreatic β cells which is characterised by mild to moderate hyper glyceamia, glucosuria, polyphagia, hypoinsulinemia, hyper lipidemia and weight loss (Hafei *et al.,* 2003). Diabetic-specific microvascular disease is a leading cause of blindness, renal failure, nerve damage and diabetes accelerated atherosclerosis leads to increased risk of myocardial infarction, stroke and limb amputation. Four main molecular mechanisms have been implicated in glucose mediated vascular damage (DCCT-1993). The concentration of protein was found 2.5µg per 10 µl by Lowry's method.

SDS Polyacrylamide gel electrophoresis:

The protein was prepared from diabetes induced rats and it was separated by SDS polycrylamide gel electrophoresis. The gel was stained with coomasive brilliant blue and bands were observed by gel documentation system. The 182 kDa protein was identified by comparing with commercially purchased protein marker. It was shown in Figure 2.To determine the role of 182 kDa protein for the induction of diabetes. We have initially monitored the development of diabetes in animals which were injected with streptozotocin.

Conclusion:

Since the diabetic rat heart showed necrosis as a secondary effect being diabetic as primary,the necrosis of the experimental animals wasanalyzed by taking heart weight,body weight ratio.Diabetes mellitus was induced byinjecting Streptozotocin(50mg/kg body weight) to rat continuosly for five days.Regular monitoring of blood glucose levels by orthotoludine method checked the induction of diabetic condition.Purified 182 kDa protein was tail vein injected to the diabetic rat(25µg) in first and third days was a result,the necrotic heart gained weight 85% compensation were observed and this was confirmed again by body weight and heart weight ratio in rats.In order to bring down myocardial infarction,our desired protein namely 182 kDa was observed in the serum and it was confirmed by Western blot analysis.

Experiment results confirmed the association of 182 kDa protein and its role in diabetes defined that,it was a stress induced protein which may confine to cardiac disease because necrotic heart is a secondary effect of diabetic mellitus.To conclude,the 182 kDa protein that may play indispensable role in the development of cardiac hypertrophy in experimental diabetic rats and may be an isoform of cardiac specific α_2 macroglobulin belonging to same macroglobulin family.Many diabetic patients suffer

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from cardiomyopathy even in the absence of vascular disease.

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