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EXPERIMENTAL MORPHOLOGICAL STUDY OF REPEATED INJECTIONS OF GLYCOSYLATED ALBUMIN

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ABSTRACT

Background and objectives: *The pathogenesis of glomerular lesions of late diabetic nephropathy is not completely understood. Glycosylated proteins are known to induce wide spectrum of diabetic changes.* ***Method:*** *A total of 36 Guinea pigs were studied in the experiment. The whole study was broadly divided into 3 sets- Experiment (A) received glycosylated albumin. Experiment (B), received non-glycosylated albumin and Experiment (C) received normal saline, injections at weekly intervals.* ***Results:*** *The test Group A, animals were alive for 12 months, showed evidence of focal thickening of basement membrane with increase in mesangial matrix. The test Group B, animals died within few hours after second injection and showed changes consistent with anaphylactic shock. However there were no specific changes in animals of test Group C.* ***Conclusion:*** *Injection of glycosylated albumin induces pseudo diabetic microvascular changes. Glycosylated egg albumin appeared to offer a protective role against Anaphylactic shock.*

KEYWORDS

Albumin, Anaphylactic, Glycosylated, Mesangial.

INTRODUCTION

The concept that some aspects of diabetic milieu leads to the development of microvascular and macrovascular changes, has received ever growing support. In particular, basement membrane thickening is an extra metabolic constant accompaniment of diabetes mellitus, due to some co-inherited propensity. Macroangiopathy, affecting the vessels of lower extremity, leading to vascular insufficiency, ischemic disease of various organs are well recognized in diabetes mellitus.

Recently, it has been suggested that basement membrane thickening in diabetes mellitus is due to the deposition of glycosylated proteins in vessel walls. Support to this data comes from the study of BA Mc Varry et al. in 1980[1] who by electron microscopic study documented pseudo diabetic renal glomerular changes in mice after repeated injections of glucosylated proteins at 12 weeks period. However, Jeraj et al [2] could find no excess glycosylated albumin accumulating after its intravenous injection. Hence, the patho-physiologic role of glycosylation of both glomerular protein accumulation and glomerular basement membrane thickening is unsettled. But glycosylation might augment this expansion.

With this background knowledge, the present study was attempted to know whether glomerular basement membrane thickening could be produced under light microscopy, with a longer injection schedule of glycosylated proteins in animals.

MATERIALS AND METHOD

Cavia Porcellus L or Male Guinea Pig Weighing 100-200gms were used in the experiment. The animals were divided in groups according to the experimental design.

Group A: 16 Guinea pigs received Glycosylated Albumin.

Group B: 14 Guinea pigs received Non-Glycosylated Albumin.

Group C: 6 Guinea pigs as control group.

Main Reagents:

Albumin: Protein powder from egg albumin was used.

1. Non-Glycosylated Albumin: 0.5gms of egg albumin dissolved in 37.5ml of sterile distilled water, under aseptic precautions. Stored at -10^{0}C until use.

2. Glycosylated Albumin (Non-enzymatically glycosylated albumin): 0.5gms of egg albumin was dissolved in 37.5ml of sterile distilled water, under aseptic precautions. To this 25ml of 25% dextrose was added. Mixture was incubated at 37^{0}C for 48hrs. Stored at -10^{0}C until use.

3. Control: 0.5ml of sterile normal saline.

These solutions were given at weekly interval to the respective animal groups.

EXPERIMENTAL DESIGN

Ethical clearance was obtained from the institutional ethical committee.

Thirty six Guinea pigs were inoculated in the protocol and divided into groups.

Experiment I (Group A)—16 animals received Glycosylated Albumin at weekly interval for 12 months.

Experiment II (Group B)—14 animals received Non glycosylated albumin at weekly intervals.

Experiment III (Group C)—6 animals received normal saline weakly intravenously for 12 months.

Animals were observed daily for survival, weight gain, gross abnormality, bleeding etc. Blood sugar was estimated before and after the experiment to rule out spontaneous diabetes mellitus or hypoglycemia. Animals which died during the course of study were autopsied; other animals were killed by cervical dislocation.

Histopathological Examination:

After going a through autopsy, representative sections were processed. H &E and PAS stains were performed. Histopathologic changes in various organs of animals belonging to different groups were evaluated and compared. An attempt was made to explain the significance of the observed findings and its possible correlation. Due to the financial constraints electron microscopy was not attempted.

OBSERVATION

Group A:

All animals showed no significant change in behavior immediately after the glycosylated albumin injection.

Test Group A showed progressive increase in weight, as compared to control group. Mean blood sugar levels in all the three groups were same before and after the experiment, i.e. 100 mg % (80-115mg %). Estimation of Glycosylated albumin was done and was 2.24 mmol/lit (1-1.25mmol/lit).

Autopsy finding: All the organs were slightly enlarged as compared to control group.

3 months and 6 months: showed no significant changes.

9months:

Lungs- showed focal dilatation of alveoli.

Myocardium- occasional vacuolation

Liver- mild fatty change

Kidney- showed focal thickening of glomerular basement membrane and mild increase in mesangial matrix.

12 months:

Lungs- Focal dilatations of alveoli, congested inter alveolar capillaries and scanty lymphocytic infiltration in the interstitium.

Myocardium- showed occasional focal vacuolation.

Liver- showed mild to moderate fatty change (**Fig.1**).

Kidney- showed patchy and focal thickening of glomerular basement membrane, which was confirmed by PAS stain (**Fig.2**). Mesangial matrix was moderately increased (**Fig.3**). Tubules showed mild cytoplasmic vacuolation. There was scant lymphocytic infiltration in the interstitium.

All the remaining organs were unremarkable.

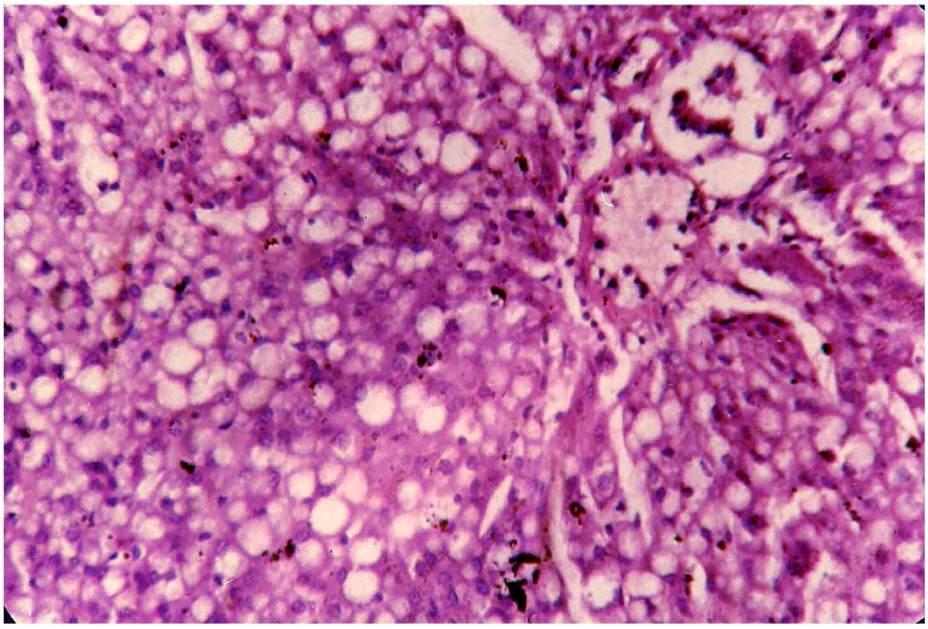

Figure.1: (Group A) section of the liver showing fatty change. (X400, H&E stain)

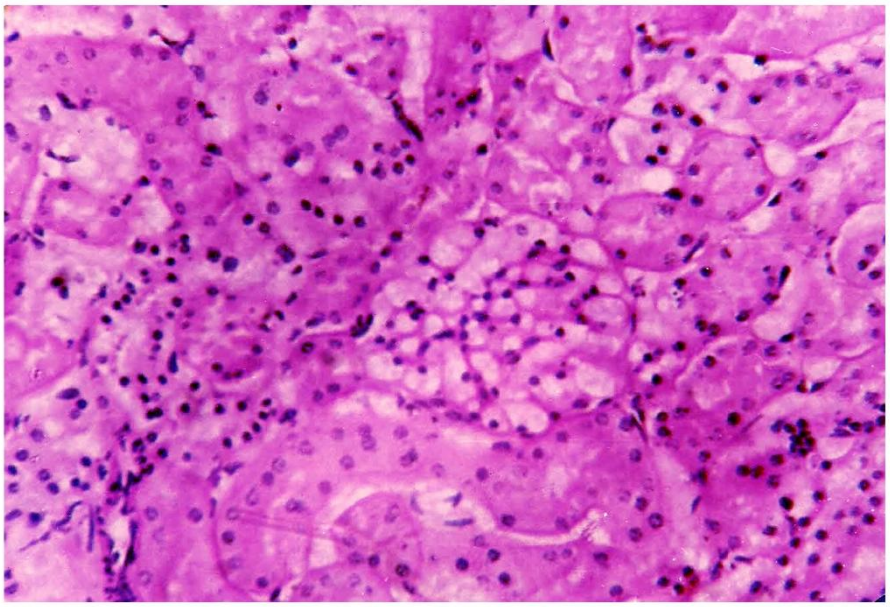

Figure.2: (Group A) section of kidney showing patchy thickening of glomerular capillary basement membrane. (X400, PAS stain)

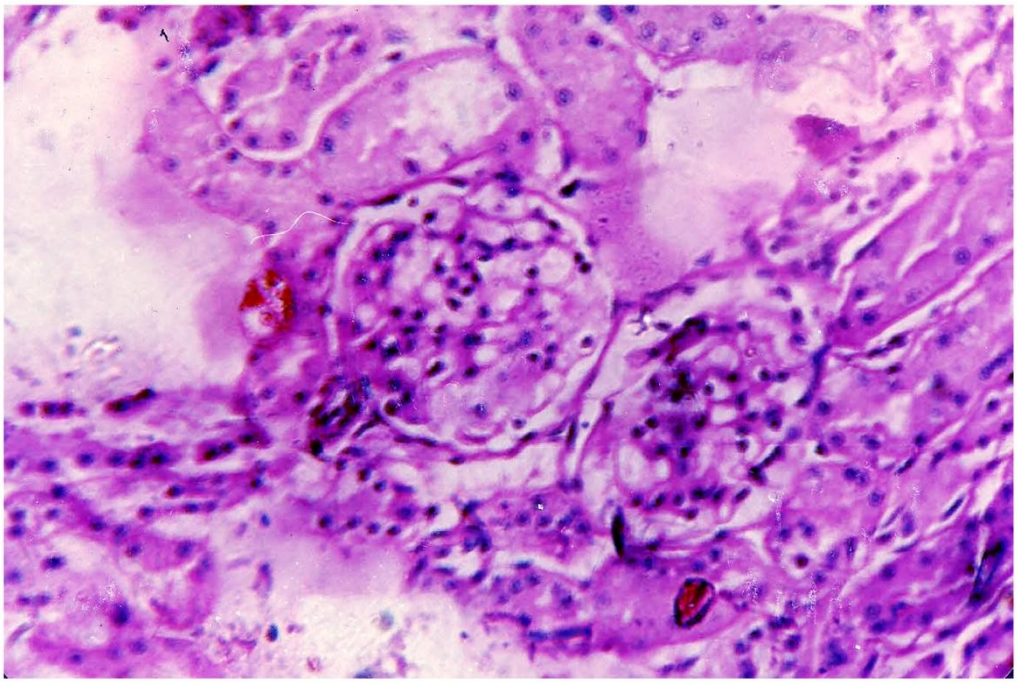

Figure.3: (Group A) section from kidney exhibiting mild mesangial sclerosis and focal hypercellularity of glomeruli (x400, PAS stain)

Group B:

All the animals in this group were dull and lethargic, after the second injection. They died within few hours due to respiratory distress. All were pale and cyanosed.

Autopsy findings: All the organs were normal in weight.

Lungs- Were heavy, firm, red and boggy. There was patchy dilation of alveoli and inter-alveolar septa. Scanty interstitial lymphocytic infiltration, edema especially around the bronchial tree and congested vessels were noticed.

Heart: few myocardial fibers showed wavy fibers and sub-endocardial,sub-epicardial hemorrhages and necrosis.

Liver- showed centrilobular congestion and marked dilation of central vein.

Spleen- showed mild congestion of red pulp.

Kidney- cortex pale and medulla congested. Focal tubular necrosis with focal edema, scant lymphocytic infiltration and congested vessels.

Gastrointestinal tract- showed patchy mucosal hemorrhages and necrosis.

The mode of death of animals immediately after second injection and the autopsy findings were suggestive of anaphylactic shock.

Group C:

The control group appeared normal in behavior and appearance.

Autopsy findings: All the organs were within normal limits (**Fig.4**).

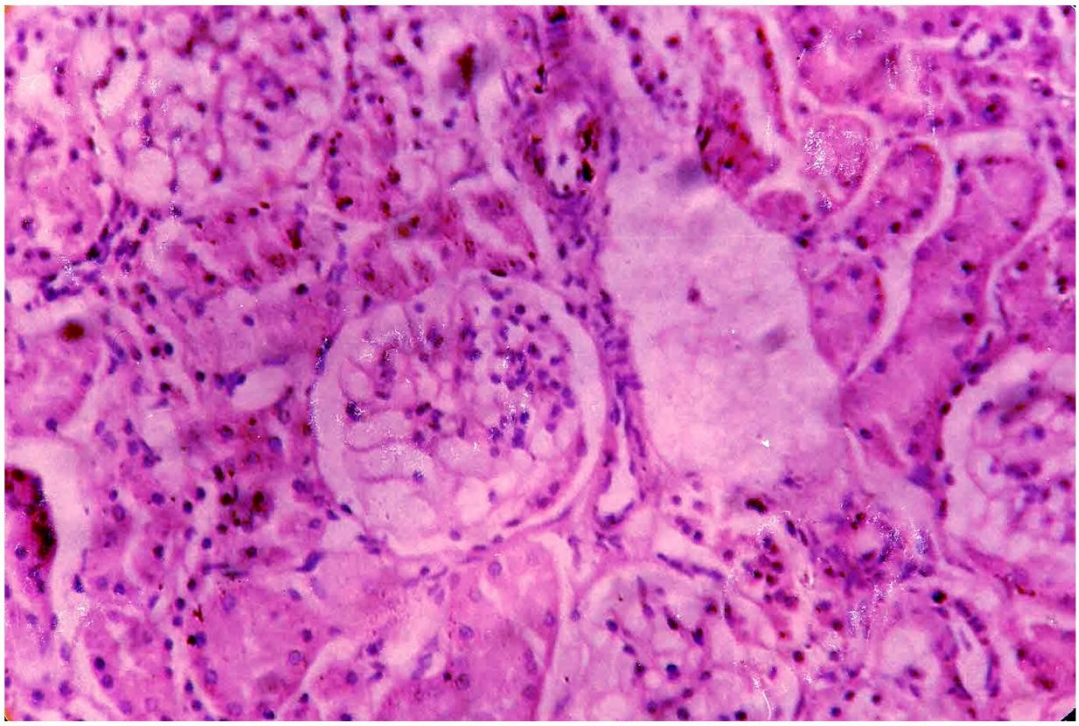

Figure.4: (Group C) section of kidney showing normal capillary basement membrane (x400, PAS stain)

DISCUSSION

Repeated injections of glycosylated proteins lead to pseudo diabetic electron microscopic changes in mice. Thus we undertook this study to see whether light microscopic pseudo diabetic changes could be induced in glomerular capillaries after prolonged injections of glycosylated egg albumin over a period of 12 months, in animals.

Glucose binds to an assortment of proteins non-enzymatically, via a process termed glycosylation. Numerous cellular proteins are modified in this manner, including hemoglobin, components of the crystalline lens, and cellular basement membrane proteins. The initial glycosylation products (known chemically as Schiff bases) are labile and can dissociate rapidly. With time, these labile products undergo complex chemical rearrangements to form stable advanced glycosylation products, consisting of a glucose derivative covalently bound to the protein amino group. As a result, the structure of the protein is permanently altered and its function may be affected [3].

The diffuse lesion consists of widespread increase in eosinophilic, periodic acid Schiff (PAS)-positive material within the mesangium[4]. Glycosylation end products (AGEs) are believed to play a role in the pathogenesis of the lesions in diabetic nephropathy. They also induce long term tissue changes like cataract, retinopathy, peripheral neuropathy and increased susceptibility to infection.

In the test group A, animals received glycosylated albumin injections for a period of 12 months. After repeated injections, there was a substantial increase in the glomerular basement membrane thickening. The results are consistent with the view that the complications of diabetes mellitus may be related to the glycosylation which occurs roughly in proportion to the severity of hyperglycemia.

There was sufficient increase in the mesangial matrix. A disturbance in mesangial phagocytic

function or an increased demand for processing of macromolecules could lead to accumulation of molecules [5]. Macromolecular protein material is disposed of inefficiently in diabetic animals.

In addition to renal glomerular changes, in present study, mild to moderate fatty change in hepatocytes and occasional mild fatty change of myocardium was also seen. Fatty liver is documented in diabetes mellitus. Studies on lipoprotein metabolism and atherogenesis in diabetes mellitus, have documented glycosylation of apoprotein B, LDL and HDL[6]. It is suggested that glycosylated albumin, may coat/combine with apoprotein, decreasing its effectiveness, or impair lipoprotein secretion from liver by coating/combining with lipoprotein and thereby may explain fatty change in group A.

In the test group B, which received non-glycosylated albumin, all the animals succumbed to death within few hours of second injection. The cause of death was possibly anaphylactic shock as evidenced by the gross and microscopic findings.

However all the animals in group A, were alive for 12 months. This possibly could be attributed to the protective effect of glycosylated albumin from anaphylactic shock. It may be that:

1. glycosylation alters the antigens or sensitogenic potential of egg albumin to render it non effective for anaphylaxis and/or

2. glycosylated albumin may coat or inhibit mast cells and prevent the release of primary and secondary chemical mediators which mediate anaphylactic shock.

3. Or glucosylated albumin may act together at various phases. Such a hypothesis needs to be substantiated with proper study.

Are the diabetics less prone for anaphylactic type of shock is a matter yet to be elicited firmly.

TO CONCLUDE

Injection of glucosylated albumin induces pseudo diabetic changes like, glomerular capillary basement membrane thickening, increase in mesangial matrix, and fatty change in liver and heart, in guinea pigs. Glycosylation of proteins in diabetes mellitus has got a possible role in the development of diabetic micro-angiopathy. Egg albumin when injected alone induces anaphylactic shock in animals like guinea pigs. Glycosylated egg albumin perhaps appeared to offer a protective role in anaphylactic shock. Lastly, this study further stimulates the profound exploration of effects of glycosylated albumin on the various systems of the body, in order to understand the pathophysiology of diabetes mellitus in proper perspective and in the prevention/treatment of diabetes mellitus and its complications.

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