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"Potential role of RAGE protein expression in platelets and monocytes among the population with diabetes mellitus."

M. Ravi Kumar[1], K. Satya Narayana[2], Anija Uchuru[3], Ivvala Anand Shaker[4]

ABSTRACT:

Background: Recent evidence suggests that RAGE may be a potential biomarker of diabetic vascular complications. The present study **aimed** to investigate the presence of RAGE in diabetes since RAGE up-regulation can be induced by AGEs. The two cell types chosen in this study were platelets and monocytes, which were isolated from human Type 2 diabetic subjects.

Materials & Methods: Twenty Five newly diagnosed type 2 diabetic patients (13 males and 12 females) in the age group of 35-50 years were taken as case from our OP. As control, 25age matched healthy volunteers (13 males and 12 females) were recruited. The conducted investigations are RAGE levels in platelets and monocytes in human diabetic subjects

Results:- The present study showed that human Type 2 diabetic subjects have significantly increased monocyte RAGE levels compared with human control subjects. RAGE levels in platelets were slightly increased but it is not significant.

Conclusion:- It is clear that AGE-RAGE interaction plays a critical role in diabetes, a better understanding of RAGE expression in platelets and monocytes may provide a better means to understand diabetic vasculopathy**.** The ability of NSAIDs to down-regulate RAGE may be of importance in the management of diabetic complications.

Keywords: RAGE (Receptor for Advanced Glycation End products); (DM) Diabetes Mellitus; AGEs (Advanced Glycation Endproducts); Hb A_{1c} – Glycated hemoglobin.

2 Department of Medical Biochemistry, Bharath University, Selaiyur 600 073

Chennai, Tamil Nadu, India.

1&3 Dept of Biochemistry,

Sakshi Medical College & Research Centre, Myana -473 001, MP, India.

4 PhD Guide, Department of Medical Biochemistry, Bharath University, Selaiyur,

Chennai-600 073,Tamilnadu, India.

Corresponding Author: Dr.Anija Uchuru

Email id: dr_anija@rediffmai.com
Mobil No: +91(0) 9849479700

INTRODUCTION : The strong association between hyperglycemia in diabetes mellitus and the development of chronic diabetic complications including both micro and macro vascular dysfunction has been shown in the results of an intervention study in both Type 1 (Diabetes Control and Complications Trial Research Group 1993) and Type 2 (UKPDS 1998) diabetic patients. Despite much research, the mechanisms by which chronic hyperglycemia results in functional changes and tissue injury are yet to be clarified, although several mechanisms have been proposed. These include enhanced glucose metabolism through the Polyol pathway, activation of PKC by hyperglycemia, increased

oxidative stress, enhanced inflammation and increased formation of AGEs.

FORMATION OF AGES :

AGEs can be found in plasma and various cell types including monocytes, red blood cell, vessel walls and kidney. Of the large number and complex family, carboxyl methyl lysine (CML) (1) and pentosidine (2) are the predominant AGEs found in human. Although a hyperglycemic environment favors the formation of AGEs. AGE cross-linking affects the biochemistry of proteins such as reducing enzyme activity (3), changing biophysical properties of proteins and affecting proteininteractions with other enzymes (4&5). RAGE plays apivotal role in the accelerated vascular dysfunctionobserved in diabetes.

Receptors for RAGE:

RAGE is a member of the immunoglobulin superfamily of cell surface molecules. Immunohistochemistry and *in situ* hybridization studies have shown that RAGE is expressed in endothelial cells, vascular smooth muscle cells, macrophage, T-lymphocytes, mesangial cells and astrocytes (6). There is a high degree of RAGE homology in human, rat and cow. The slight differences in glycosylation sites and susceptibility to proteases may contribute to their different pharmacological parameters (7 & 8).

Cellular interaction of AGEs: central role of RAGE in diabetes :

In the presence of diabetes, cells and tissues are constantly exposed to AGEs from the circulating blood and consequently results in AGE accumulation. The cross-links between AGEs and proteins/tissues can lead to disturbances in vascular homeostasis. For example, in a diabetic rat model following AGE-RAGE engagement, endothelial cells reduce in size resulting in increased endothelial permeability (10), which is an early sign of the development of diabetic vasculopathy. **MATERIALS AND METHODS:**

RAGE LEVELS IN PLATELETS AND MONOCYTES IN HUMAN DIABETIC SUBJECTS

Human subjects and blood sample collection (Type 2 diabetes)

Twenty Five newly diagnosed type 2 diabetic patients (13 males and 12 females) in the age group of 35-50 years were taken as case from our OP. As control, 25age matched healthy volunteers (13 males and 12 females) were recruited. None of the subjects were receiving any form of drugs. Subjects with the habit of smoking and taking alcohol were also excluded from the study. All the experimental procedures were approved by the Institute Human Ethics Committee and informed consent was obtained from all the participants.

Isolation of human platelets and monocytes

The isolation of human platelets and monocytes was performed using OptiPrepTM solution according to the manufacturer's protocol.

Protein assay

Determination of human sample protein concentrations was performed using the Biorad microBCA system.

Western blot: RAGE protein

Human samples were loaded in duplicate with a final total protein concentration of 10 µg per

well. The protein samples and molecular weight markers were electrophoresed, transferred onto nitrocellulose membranes and incubated with anti-mouse monoclonal anti-RAGE antibody using the Western blot protocol.

DRUGS AND CHEMICALS

Anti-mouse monoclonal anti-RAGE antibody, peroxidase conjugated goat anti-mouse immunoglobulins; Chemicon International.

STATISTICAL ANALYSES:

Differences in the percentage densities of Western blot bands were analyzed using unpaired Student's *t*-test, Mann-Whitney test, 1-way ANOVA and post-hoc tests (Tukey's, Games-Howell and Dunn's multiple tests), Pearson Chi-Square and Fisher's exact test whenever applicable. **Specificity of Western blot: RAGE (humans)**

RAGE protein bands were determined and verifiedagainst their corresponding molecular weight at approximately 48 kD. The specificity of the primary RAGE antibody was further assessed by incubating samples with the secondary antibody only.

DISCUSSION:

Non-enzymatic glycosylation macromolecules such as proteins, enzymes and lipids results in the formation of irreversible AGEs (11 &12). The consequence of AGE formation is reduced enzyme activity, alteration in protein structures and altered protein interactions (13-15). Increasing evidence suggests that the engagement of AGEs to specific receptors such as RAGE plays a pivotal role in the accelerated vascular dysfunction observed in diabetic animal models (16 &17). The present study investigated the presence of RAGE in diabetes since RAGE up-regulation can be induced by AGEs (18 & 19). The two cell types chosen in this study were platelets and monocytes, which were isolated from human Type 2 diabetic subjects.

RAGE LEVELS IN TYPE 2 DIABETES

Platelet RAGE in Type 2 diabetes

There is clear evidence of platelet hyperactivity in Type 2 diabetes, but it is unclear whether increased RAGE levels in platelets could play a role since platelets are devoid ofgenetic material and the most prominent RAGE actions are related to changes in gene expression (20). There is evidence for enhanced expression of proteins such as P- selectin, CD 40 ligand, and receptors such as thromboxane receptor α in platelets, which are pre-formed and stored in the α granules in platelets. Upon activation, these proteins/receptors are then redistributed to the surface of the platelets (21 &22).

Monocyte RAGE in Type 2 diabetes

The present study showed that human Type 2 diabetic subjects have significantly increased monocyte RAGE levels compared with human control subjects. In the literature, several studies reported that RAGE was up- regulated in diabetes. For example, Buchs et al. (23) observed increased RAGE expression in peripheral blood mononuclear cells of human Type 2 diabetic subjects with vascular complications compared to human diabetic subjects without complications. Other studies also observed increased RAGE levels in various tissue/cell types. For example, RAGE was up-regulated in endothelium, VSMCs, monocyte-derived macrophages and cardiac myocytes of a bovine diabetic model (24). Enhanced RAGE expression may be crucial to the development of atherosclerosis in diabetes. Several animal studies have reported that treatment of diabetic mice with advanced atherosclerosis with soluble RAGE (a RAGE inhibitor) suppressed the development of atherosclerosis (25 &26).

Monocyte infiltration and conversion to macrophages are pivotal steps in atheroma formation and hence monocyte RAGE may be important. The engagement of RAGE-bearing monocytes by AGE ligands can lead to chemotaxis (18) and monocyte infiltration to the endothelial monolayer (20-24). In atherosclerotic plaques isolated from humanType 2 diabetic subjects, Cipollone et al. (27) observed an enhanced macrophage and T cell infiltration compared to non-diabetic plaques accompanied by increased RAGE expression. Further immuno-staining showed that active macrophages in these plaques actually expressed RAGE. Cipollone and colleagues further reported increased activation of NF-κB in diabetic plaques and this showed a strong concordance with RAGE expression, althoughfurther studies are needed to determine a directrelationship. Interestingly, increased RAGE expression in monocytes may potentially link to COX-mediated pathways as Cipollone et al. (27) found that increased RAGE was co-localized with increased COX-2, PGE synthase and MMP expression in the activated macrophages derived from diabetic atherosclerotic plaques. Another study by Bucciarelli et al. (28) showed that COX-2antigen levels were reduced in the aorta of diabetic mice in the presence of soluble RAGE.

CONCLUSIONS:

It is clear that AGE-RAGE interaction plays a critical rolein diabetes, a better understanding of RAGE expression in platelets and monocytes may provide a better means to understand diabetic vasculopathy**.** The ability of NSAIDs to down-regulate RAGE may be of importance in the management of diabetic complications.

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