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Genetic variant of *RAGE* gene and its enhanced expression: Risk factor for vascular complications in type 2 diabetes mellitus

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Background & Aim: Advanced glycation end products (AGEs) are formed as a result of spontaneous non-enzymatic glycosylation of biomolecules, like proteins, lipids, nucleic acids. Interaction of AGEs with its receptor *RAGE* induces signal transduction that culminates in vascular complications, the major cause of morbidity and mortality in diabetic subjects. Some functional polymorphism of *RAGE* gene show differential activity of this receptor and therefore may be associated with the development of vascular complications in diabetic patients. In the present study we estimated blood level of AGEs and investigated the association of expression of *RAGE* gene and its genetic variants namely -374T/A and -429T/C in the promoter region and Gly82Ser polymorphism in the exon 3 region with vascular complications in T2DM patients.

Methods: We screened 820 subjects which includes 200 healthy controls, 200 type 2 diabetes mellitus (T2DM) subjects without any vascular complications (DM), 220 T2DM subjects with microvascular complications (DM-Micro) and 200 T2DM subjects with macrovascular complications (DM-Macro) for -374 T/A, -429 T/C and Gly82Ser polymorphisms of *RAGE* gene. DNA isolated from the enrolled subjects was genotyped by PCR-RFLP. *RAGE* expression was determined by quantitative real-time PCR. Serum AGEs was estimated by spectrofluorometry.

Results: Serum AGEs level was significantly higher in diabetic patients having vascular complications as compared to T2DM without complications ($p<0.01$). Mutant variant of -429T/C and Gly82Ser *RAGE* polymorphism was about three times more prone to develop macro vascular and micro vascular complications respectively in T2DM subjects while -374A allele showed reduced risk towards the development of macro vascular complications (OR=0.57, p=0.006). Further, haplotype analysis revealed that CTG haplotype was significantly associated with the development of macro vascular complications while haplotype TAG was observed to be significantly protective towards development of macro vascular complications in T2DM subjects. The expression of *RAGE* correlated significantly with the genotypic variation of the *RAGE* gene.

Conclusion: Mutant genotypes of *RAGE* gene and enhanced formation and accumulation of AGEs under hyperglycemic conditions enhance *RAGE* expression in diabetic patients causing increased AGE-*RAGE* interaction, may be considered as risk factor for vascular complications in North Indian T2DM patients.

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In vitro toxicity screening of poly phyto combination using Caco-2-cell line

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The aim of the research work is poly phyto combination compression of different anti-lithiogenic agents and to screen its toxicity by Caco-2-cell line. Polyphyto combination was prepared by geometrical dilution method; it was subjected for aqueous and alcoholic extract. The formulation and extract were screened for its initial *in vitro* toxicity study using Caco-2-cell line by 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay method for aqueous extract of formulation. It showed nontoxic to Caco-2-cell line, even at high dose, that is, 1000 µg/ml, whereas aqueous extract (FH1) exhibited moderate toxicity CTC 50 value 875±35.3 µg, FH1 crude formulation showed significant toxicity against Caco-2-cells with low concentration with CTC 50 value 382.75 based on this result the conclusion was drown that the formulation FH2 and aqueous extract of FH1 is devoid of toxicity. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS).

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