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Original Research Article

Angiopoietin 2 in type 2 diabetes mellitus patients and those with complications: an observational comparative study

Kishan Raj K.*

Department of Medicine, Yenepoya Medical College, Mangaluru, Karnataka, India

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***Correspondence:**
Dr. Kishan Raj K.,
E-mail: kollampareraj@gmail.com

ABSTRACT

Background: Angiopoietin 2 levels in blood signifies neo vascularization. Only biomarker available now for routine check up is HbA1c. However, it doesn't suggest if patient is more prone to get into complications than the other. Here, we try to bring in another biomarker Angiopoietin 2 and elucidate if it can identify patients going in for complications of type 2 diabetes mellitus early.
Methods: Total 60 diabetic patients were studied over a span of 1 year. 30 were diabetic without any complciations and another 30 were with complications (Diabetic foot ulcer, Diabetic retinopathy, Diabetic nephropathy, Diabetic neuropathy). Angiopoietin 2 levels were estimated in both the groups and compared.
Results: Analysis showed Mean duration of Diabetes was significantly lower in patients without complication than those with complications. Human angiopoietin 2 levels were elevated in both the study groups. More so, in the study group with Diabetic patients with complications. It was statistically significant. ($p<0.001$).There was no significant relationship between duration of diabetes and Human angiopoietin 2 levels.
Conclusions: It was found that angiopoietin 2 is also a good biomarker for diabetes as HbA1c. It also helps in detection of complications earlier and thus may help in reducing morbidity as well as mortality. Further studies are required to strengthen the information got from this study to compare efficacy of HbA1c and angiopoietin 2 as well as how early can we detect the patients who may land up in complications.

Keywords: Angiopoietin 2, Complications of Type 2 diabetes mellitus, Type 2 diabetes mellitus

INTRODUCTION

Diabetes clinical trials have long relied on biomarkers for proving efficacy of study drugs. Although hemoglobin A1c (HbA1c) was first separated from other forms of hemoglobin by Huisman and colleagues in 1958 using a chromatographic column, its use for monitoring the degree of control of glucose metabolism in diabetes patients was first proposed in 1976 by Koenig and coworkers.[1,2] It is clear that HbA1c is far from a perfect biomarker. For example, levels of postprandial glucose, as well as acute fluctuations in glucose levels, may be better predictors of cardiovascular disease in diabetes compared with levels of HbA1c.[3,4]

Hence there is a need for studying of newer biomarkers which can overcome the shortcomings of HbA1c. Angiopoietin-2 promotes cell death and disrupts vascularization. Yet, when it is in conjunction with vascular endothelial growth factors, or VEGF, it can promote neo-vascularization.[5]

Angiopoietin-2 is produced and stored in Weibel-Palade bodies in endothelial cells and acts as a TEK tyrosine kinase antagonist. As a result, the promotion of endothelial activation, destabilization, and inflammation are promoted. Thus, can be used as a biomarker in detecting neovascularisation and early complication in Diabetes Mellitus patients.

METHODS

Justification for this study

This type of study has not been conducted in India. Angiopoietin 2 can emerge as a novel biomarker for T2DM with further research on this topic. Drugs targeting Ang1/Ang2–Tie2 can be used as an adjuvant treatment in T2DM.

Inclusion criteria

- Established T2DM (acc. To WHO definition).
- Aged 18-80 years (both males and females).
- Established cases of T2DM with complications (Diabetic retinopathy, diabetic nephropathy, diabetic foot, diabetic neuropathy).

Exclusion criteria

- Patients who did not consent for the study.
- Gestational Diabetes Mellitus were excluded.
- Patient with serious comorbidities (Sepsis, Liver disease, seriously ill patients).
- Type 1 Diabetes mellitus.

Study duration was of 1 year. Total 60 T2DM patients all being In-patients in VMMC, Karaikal. Principle of the method includes Solid phase sandwich assay ELISA with Hu Ang-2.

Human Angiopoietin-2 ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) is designed to detect and quantify the level of human angiopoietin-2 in serum, plasma, buffered solution, tissue culture medium, and cell lysates.. The assay will recognize both natural and recombinant human angiopoietin-2. Perform sample dilutions with Standard Diluent Buffer. Human serum and plasma require a 10-fold dilution in the Standard Diluent Buffer (e.g., 25 µL sample into 225 µL of buffer).

Prepare 1X Streptavidin-HRP solution Perform ELISA (Total assay time: 3 hours) Read the absorbance at 450 nm. Read the plate within 30 minutes after adding the Stop Solution

Statistical analysis

Data collected under each group was summarized as Mean±SD. Statistical analysis was carried out using paired t-test for within the group comparisons and unpaired t-test for between the group comparisons. A 'P' value of <0.001 is considered statistically highly significant whereas a 'p' value of <0.05 is considered as statistically significant and a 'p' value of >0.05 was considered statistically not significant. The IBM - SPSS v21.0 (International Business Machines Corporation - Statistical Package for Social Science program version 21.0) software was used for statistical analysis of data.

RESULTS

This study was conducted on a study population of 60 of which 12 were females and 48 were males with their ages ranging from 40 years to 70 years with mean being 50±7.53.

They were classified into 2 groups each group consisted of 30 subjects each:

- Group A: Study subjects with Type 2 diabetes but without complications
- Group B: Study subjects with Type 2 Diabetes Mellitus with complications which included Diabetic nephropathy, neuropathy, retinopathy, Diabetic foot ulcer.

Human angiopoietin 2 levels were elevated in both the study groups. More so, in the study group with Diabetic patients with complications. It was statistically significant (p<0.001).

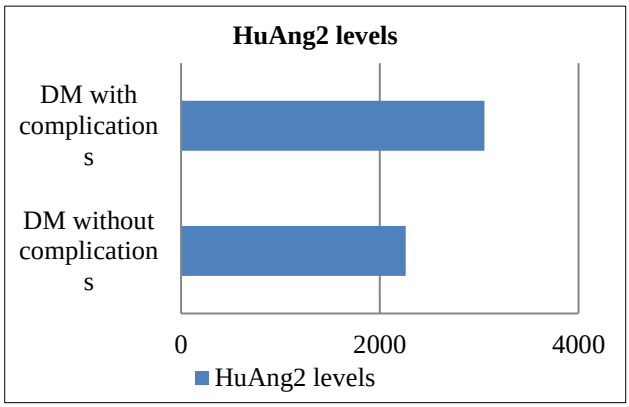

Figure 1: Angiopoietin 2 levels in patients with diabetes with and without complications.

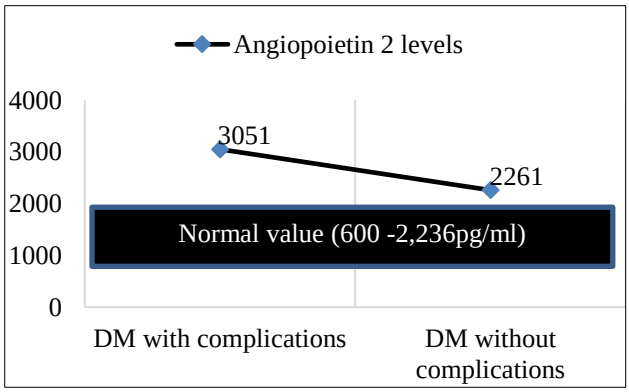

Figure 2: Comparison of angiopoietin 2 levels in patients with type 2 diabetes mellitus with and without complications and comparison of the same with normal value.

Angiopoietin 2 levels were higher in patients with Diabetes with complications than those without

complications. Both the groups had angiopoietin 2 levels above the normal range.

DISCUSSION

Type 2 diabetes mellitus is the predominant form of diabetes worldwide accounting for 90% of cases, it has become one of the world most important public health problems and it is now well established that the 21st century will be characterized by a global epidemic of it.[6]

The prevalence of type 2 diabetes mellitus is rising at an alarming rate throughout the world.

Identifying and treating complications which was the paradigm of treatment before has now slowly shifted to prevention of complications by stringent control of sugar values in early stages of detection.

This can be achieved with the help of biomarkers which help us know if a patient is going in for complications and who need more care and need strict diabetic control.

Angiopoietins are vascular growth factors involved in angiogenesis and vasculogenesis. Two isoforms of much importance are: Ang-1 and Ang- 2, both ligands for the Tie-2 receptor, found primarily on endothelial cells and podocytes.[7]

Angiopoietins can promote angiogenesis, remodeling, maturation and maintenance through using a coiled-coil domain in the N-terminus and a fibrinogen-like domain in the C-terminus.[8] In addition, some angiopoietins are potent regulators of lipid, glucose, and energy metabolism.

Since no previous data on reference levels of Ang-2 in Indian adults are available, results of the present study cannot be compared with the other studies from the region.

Hyperglycemia is considered as a major cause which exerts toxic effects on the endothelium and leads to inflammation which in turns increases the production of Ang-2.

Hyperglycemia promotes toxic effect on vascular endothelium by the production and activation of advanced glycation end products (AGE) and reactive oxygen species (ROS) which leads to the development of micro vascular and macro vascular complications in diabetes mellitus.

Patients with complications of diabetes mellitus had higher angiopoietin 2 levels as compared to those without complications but as compared to a study done in Saudi by Siddiqui et al, our study did not show any changes in Angiopoietin 2 levels with age and duration of diabetes.

There was also no significant difference in BMI in patients with diabetes mellitus without complication with that of patients with complications as was observed in other studies in Saudi and Chennai.[9] Study population comprising of diabetics with complications, the most common complication was diabetic foot ulcer.

The angiopoietin 2 levels were significantly increased in patients with diabetes with complications.

Moreover, previous studies highlighted importance of diabetic nephropathy and neuropathy. Here I am emphasizing on diabetic foot ulcer as another common complication where angiopoietin 2 levels can be raised.

In the multivariable logistic regression analysis, Ang-2 levels were independently associated with T2DM patients with vascular complications, a finding which is in par with our study result as well.[10]

This indicated that the extent of vascular damage may be closely linked to circulating Ang-2 levels. Several hypotheses can be made on the role of Ang-2 levels in diabetic vasculopathy.

First, Ang-2 alone may exert pro-angiogenic activity. Second, high Ang-2 levels may result in local inflammation, as evidenced by leukocyte attachment to the wall of post-capillary venules, and increased blood vessel permeability.

Study subjects were equally divided into 2 subdivisions : one group with Diabetic patients without complications and the other group with Diabetic patients with complications.Human angiopoietin 2 levels were analysed in both groups and were compared along with other parameters which define Diabetes.

Human angiopoietin 2 levels were elevated in both the sub groups than the normal range..Human angiopoietin 2 levels were significantly elevated in Subjects with complications than those without complications. Similar change was seen in HbA1c as well. Duration of Diabetes as well was directly proportional to development of complications.

CONCLUSION

Human angiopoietin 2 levels can act as novel biomarker in identifying patients with diabetes who are under risk of developing complications in the near future and thereby prevent or help in maintaining the diabetic status without complications for a longer period of time. Larger and more robust studies are required in this particular field so as to find if Angiopoietin 2 levels alter with race, sex and other factors involved in Diabetes Mellitus. Scope for studies on drugs that help in altering Human angiopoietin 2 and thus helping Diabetic care in the future can also be tried.

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