श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, त्रिवेंद्रम, तिरुवनन्तपुरम - 695 011, केरल, भारत
SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY, TRIVANDRUM
THIRUVANANTHAPURAM - 695 011, KERALA, INDIA
(एक राष्ट्रीय महत्व का संस्थान, विज्ञान और प्रौद्योगिकी विभाग, भारत सरकार)
(An Institution of National Importance, Department of Science and Technology, Government of India)
टेलीफोन नं./Telephone No.: 0471-2443152 फैक्स/Fax: 0471-2446433, 2550728
ई-मेल/E-mail: sct@sctimst.ac.in वेबसाइट/Website: www.sctimst.ac.in

PROJECT COMPLETION REPORT

1. Project Number **5360**

2. Title of the Project **Desialylation-driven uptake of lipoprotein(a) to endothelial cells and monocytes / macrophages in diabetic cardiovascular patients: Is immune complex with natural antibodies a vehicle?**

3. Funding Agency Name **SERB**

4. Project Reference Number provided by the Funding Agency: **EMR/2016/006562**

5. Principal Investigator (Name & Address) **: Dr. Geetha M, Scientific Officer,**

Department of Biochemistry, SCTIMST

Medical College P.O, Trivandrum-695011

KERALA

6. Co-Investigators (Name & Address): **1. Dr. P. S Appukuttan**

Professor & HOD (Retired)

Department of Biochemistry, SCTIMST

Medical College P.O, Trivandrum-695011

KERALA

2. Dr. Ajit Kumar V K

Professor & HOD (Retired)

Department of Cardiology, SCTIMST

Medical College P.O, Trivandrum-695011

KERALA

7. Implementing Institution **:** **SCTIMST**
8. Collaborating Institutions**:** **NIL**
9. Date of Commencement**:** **17-07-2018**
10. Duration**:** **24 months**
11. Date of completion**:** **16-07-2020**
12. Objectives as approved**:**

1. **Isolation and characterization of plasma Lp(a) immune complex in order to assess desialylation status of Lp(a)in CVD as well as diabetic CVD patients and comparison to normal healthy individuals**
2. **Elucidation of relevance of molecular size and concentration of Lp(a) in CVD using newer protocols for determining Lp(a) size and assay of plasma levels developed in this laboratory.**
3. **Assay of binding in vitro of desialylated Lp(a) IC to human endothelial cells /monocytes / macrophages after exposing the latter to diabetic levels of neuraminidase**

13. Deviation made from original objectives if any, while implementing the project and reasons thereof : **NIL**
14. Field/Experimental work giving full details of summary of methods adopted, data collected supported by necessary tables, charts, diagrams and photographs :

Summary of methods adopted:
10ml of fasting blood sample from three categories of volunteers was collected in vacutainer tubes for serum collection:(a) Normal individuals as healthy control volunteers (b) CVD patients with diabetes (c) CVD patients with no history of diabetes. Following experimental methods were adopted on these three types of samples

1. Serum Lp(a) Assay Using Polystyrene well-coated Jacalin (J-a Assay) : This assay was carried out by a method developed and published by this laboratory. Briefly, specified dilution of plasma was added to polystyrene ELISA wells coated with jacalin and probed with anti apo(a) antibody labeled with HRP

2. Assay of the desialylation status of Lp(a) in immune complex Lp(a)-IC in serum: Serum Lp(a) immune complex was collected by PEG precipitation method and Lp(a) in the immune complex was dissociated by urea dissociation method. The dissociated Lp(a) was collected using ultracentrifugation after density adjustment with KBr. The desialylation status of dissociated Lp(a) was ascertained using peanut agglutinin labeled with HRP (PNA-HRP)

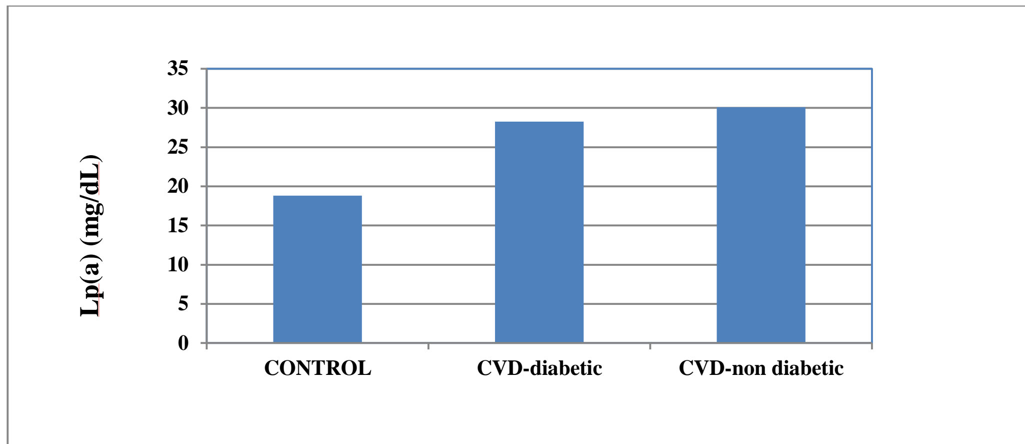
3. Ratio of mobility of LDL to Lp(a) in TBE electrophoresis: Lp(a) from plasma was subjected to TBE disc electrophoresis. The ratio of mobility of LDL to that of Lp(a) was used as an index of molecular size of Lp(a) isoforms.

4. Antibody distribution in serum Lp(a) immune complex: Polystyrene wells were coated with anti apo(a) antibody and circulating immune complex isolated by PEG precipitation was added. Immunoglobulin type distribution in Lp(a) –IC was determined by incubation with HRP conjugated anti-human IgA, IgG, IgM separately.

5. Haemagglutination by Lp(a) IC: Desialylated O-group human red blood cell was used instead of desialylated monocytes/ endothelial cells. Two fold dilutions of PEG precipitated Lp(a) –IC (100 µl) followed by 25 µl desialylated O-grp human RBC (5% v/v) was added to polystyrene wells. After mixing and incubation for 1h at 25°C, the contents were again mixed and settling of RBCs within 2 min were taken as positive agglutination in comparison to agglutination using non desialylated human o-group RBC

Data collected (Figures)

1. **Serum Lp(a) assay of control and CVD (diabetic and non-diabetic) volunteers**



<u>Lp(a) assay values</u>

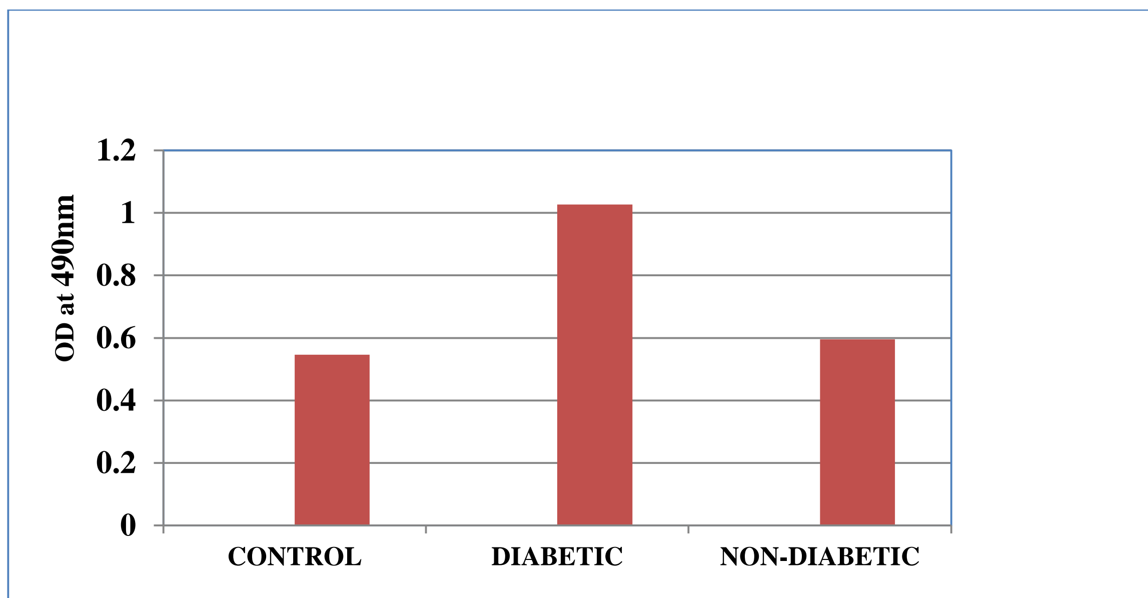
Control= 18.8 mg/dL

CVD (diabetic)= 28.2 mg/dL

CVD (non-diabetic)= 30 mg/dL

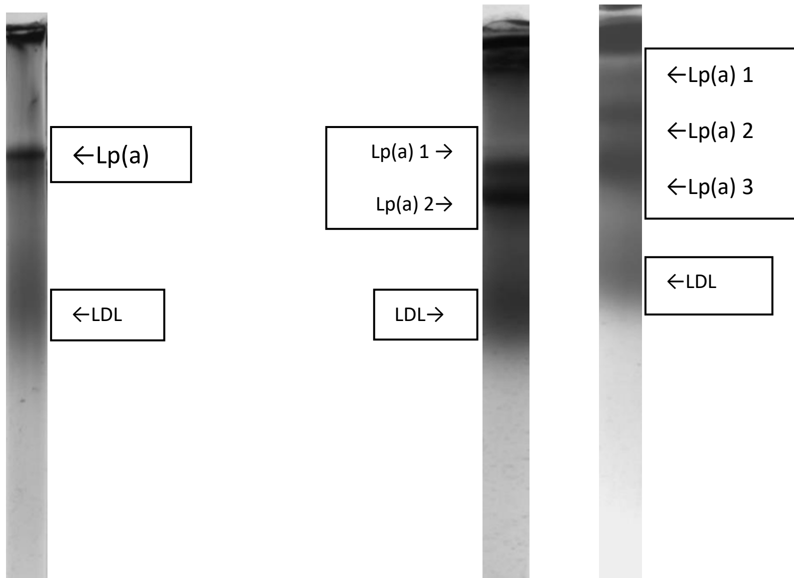
Compared to normal (control) individuals, CVD patients have 55% elevated serum Lp(a) concentration (diabetic CVD patients 50% and non-diabetic CVD patients have 60%).

2. **Desialylated Lp(a) comparison in control / diabetic CVD/ non-diabetic CVD individuals**



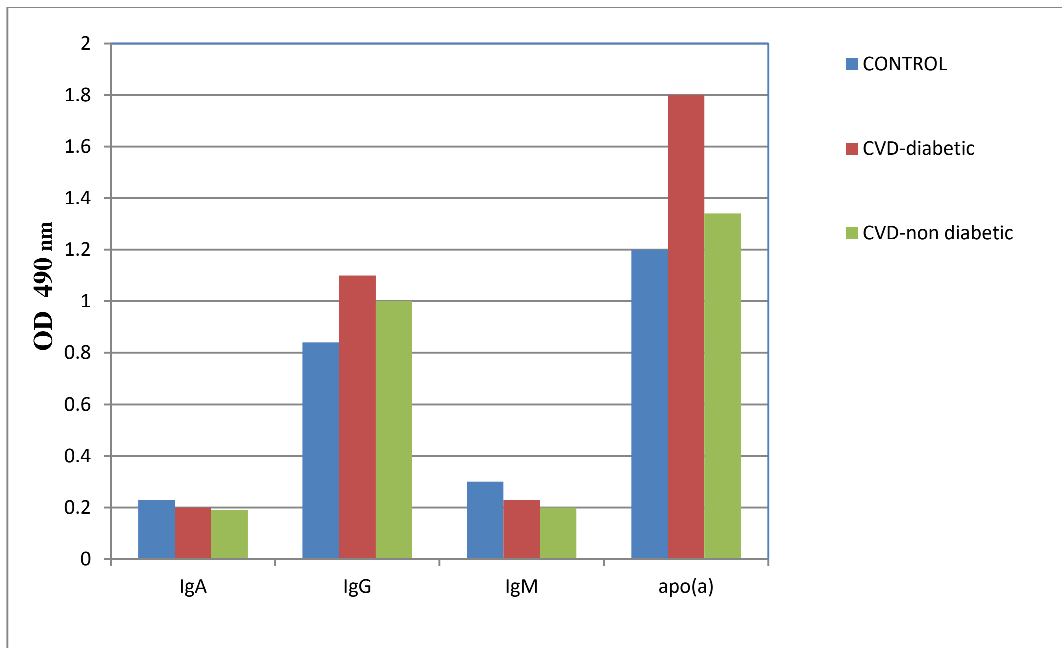
Diabetic individuals carry 88% higher levels of desialylated Lp(a) in serum compared to normal control individual whereas non-diabetic individuals carry 8.97% higher levels of desialylated L p(a) in serum

3. Ratio of mobility of LDL to Lp(a) in TBE electrophoresis



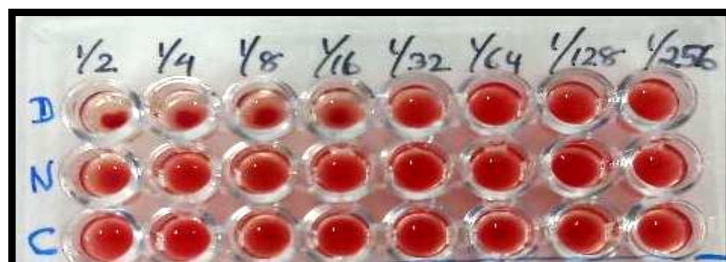
Normal volunteer's serum **CVD patient's serum (2 samples)**

4. Antibody distribution in serum Lp(a) immune complex



- **IgG** is the dominant antibody in Lp(a) –IC [IgA: **IgG**: IgM = 1: **5** :1]
- Compared to control individuals, CVD patients have **30%** higher load of Lp(a)-iC in circulation
- CVD patients with **diabetes** have **50%** and **non-diabetic CVD** have **11.7%** higher load of Lp(a)-IC with remarkable dominance of IgG antibody in both cases

5. Haemagglutination by Lp(a) IC



D- Diabetic; N-Non-diabetic; C- Control

- Lp(a)-IC from **diabetic-CVD patients** agglutinated desialylated red cells up to a dilution of **1: 16**
- Lp(a)-IC from **non-diabetic-CVD patients** agglutinated desialylated red cells up to a dilution of **1: 2**
- Lp(a)-IC from control individuals showed no red cell agglutination

15. Detailed analysis of results :

Lipoprotein (a) [Lp)a] is a circulating lipoprotein that characteristically contains an apoprotein, apo (a) which is rich in sialylated O-glycans. Elevated plasma levels of Lp(a) has been suggested to be an independent risk factor for stroke, aneurysm, cardiovascular and peripheral vascular disorders. The reported presence of more apo(a) than apoB in atherosclerotic plaques even though LDL is many times more in circulation than Lp(a), highlights the role of Lp(a) in vascular pathology. However molecular mechanisms of this discriminative uptake of Lp(a) into sub-endothelial cells has not been elucidated.

Involvement of LDL in circulating immune complex (IC) had been studied extensively but studies related to Lp(a) IC are limited. Lp(a) IC involving anti-carbohydrate antibodies anti-Gal and LIg reported from our laboratory have been observed to possess unoccupied binding sites on the antibodies involved; by virtue of which anti-Gal-Lp(a) IC binds to cognate affinity matrix cross-linked guar gum and LIg-Lp(a) IC binds to desialylated human red cells resulting in their agglutination.

Organelle dysfunction including that of lysosomes has been observed as hallmark of diabetes. Sialic acid terminals on glycoproteins and glycolipids are crucial to their stability, maintenance of cell surface charge as well as for modulating interactions with carbohydrate-recognizing molecules. The desialylating enzyme neuraminidase will also be released by damaged lysosomes. Indeed it has been reported recently that diabetes causes desialylation of membrane-bound and secreted glycoproteins apparently due to increased release of neuraminidase enzyme by damaged lysosomes into extracellular space and into circulation. Two prominent plasma immunoglobulins known to readily recognize desialylated macromolecules are LIg and anti-T which recognize N-acetyl lactosamine and Gal β 1→3GalNAc (T antigen) that get exposed following desialylation of N- and O-linked oligosaccharide side chains on glycoconjugates. Both these antigens are abundant in Lp(a). A surge in neuraminidase in plasma can therefore result in circulating Lp(a) IC in which

the antibody still contains unoccupied binding sites, due to steric reasons. These ICs can get lodged to the desialylated cells or surfaces utilizing the unused binding sites and pave way for inflammatory vascular diseases. Such IC formation may also explain the entry of Lp(a) in the sub-endothelial space since no apo(a) receptor is reported on the endothelial surface.

Hence a study involving Lp(a) IC and their desialylation status in cardiovascular patients with and without diabetes in comparison with healthy individuals may throw light on the role of diabetes in cardiovascular inflammation. Results may offer possibility of preventing IC formation and atherosclerosis in diabetics using biological glycopeptides as inhibitors of Lp(a) IC formation.

Result analysis

1. Comparison of serum Lp(a) among the three categories of volunteers viz. normal controls, cardiovascular patients (CVD) with and without diabetes shows that the mean serum Lp(a) in normal volunteers is 18.8 mg/dL whereas in CVD patients it is 29.1 mg/dL showing that there is 55% elevated serum Lp(a) concentration in CVD patients compared to the normal individuals. The Lp(a) moiety was further analysed with respect to its desialylation status is the above group of volunteers.
2. Lp(a) immune complex in circulation was collected by polyethylene glycol precipitation protocol and Lp(a) moiety in the complex was dissociated using urea. The desialylation status of the released Lp(a) was ascertained using lectin from peanut, peanut agglutinin(PNA) It is the lectin that specifically binds to desialylated core-1 O glycans and hence its binding to Lp(a) earmarks its desialylation status. Based on above protocol the present study reports that diabetic individuals carry **88%** higher levels of desialylated Lp(a) in serum compared to normal control individual whereas non-diabetic individuals carry only **8.97%** higher levels of desialylated Lp(a) in serum. Serum sialic acid and sialydase levels are already reported to be elevated in cardiovascular patients and much higher levels in diabetic individuals. As a result the cellular components, circulating moieties like Lp(a) as well as the endothelial surface gets desialylated. Humoral immune response to desialylated Lp(a) and the mechanism of its entry into the sub endothelial layer is unexplored. The finding in the present work that cardiovascular patients with diabetes carry such enormous quantity of desialylated Lp(a) explains partially the route of entry of Lp(a) into plaque. Desialylated Lp(a) has exposed T antigen and hence it is like a multivalent antigen which is easily prone to immune complex formation with natural antibodies. Such a complex formed between a multivalent antigen and antibody, extends to form a lattice having unoccupied binding sites on the antibody enabling the complex to anchor to endothelium / cell surfaces.
3. This concept was applied on red blood cells to study the difference in the anchoring capacity of Lp(a) immune complex from the three categories of volunteers participating in this study. As is shown in figure Lp(a)-IC from **diabetic-CVD patients** agglutinated desialylated red cells up to a dilution of **1: 16.** Lp(a)-IC from **non-diabetic-CVD patients** agglutinated desialylated red cells up to a dilution of **1: 2 whereas** Lp(a)-IC from control individuals showed no red cell agglutination at a dilution of 1:2. The above results clearly show the heavy load of Lp(a)-IC with anchoring capacity present in CVD patients with diabetes. CVD patients with no diabetes have relatively low or negligible Lp(a)-IC with red cell anchoring capacity. Study was further extended to know the type of antibodies present in Lp(a)-IC.
4. Since it is already reported by in vitro studies from this laboratory that Lp(a) forms immune complex with natural antibodies like anti-Gal and LIg, it was thought necessary to find the general

distribution of IgG, IgA and IgM in the circulating Lp(a)-IC. Results revealed that IgG is the dominant antibody in Lp(a) -IC; the relative antibody ratio being IgA: IgG: IgM = 1: 5:1**.** Assay of Lp(a)-IC showed that CVD patients have **30%** higher load of Lp(a)-IC in circulation compared to normal control volunteers. Comparison between diabetic and non-diabetic CVD patients showed that patients with **diabetes** have **50%** and **non-diabetic CVD** have **11.7%** higher load of Lp(a)-IC with remarkable dominance of IgG antibody in both cases. The natural antibodies anti-Gal and LIg reported to bind to Lp(a) by in vitro experiments constitutes mainly of IgG and hence it may be possible that the heavy load of Lp(a)-IC present in circulation of CVD patients consists of Lp(a) complexed with these natural antibodies. These complexes consisting of a heavily glycosylated Lp(a) molecule and antibodies enable them anchor to cells and surfaces provided they have free binding sites. Agglutination of desialylated human cell taken as a model proves that these complexes have free binding sites.

5. The molecular size of Lp(a) and its relation to CVD had always been a subject of debate. High serum Lp(a) concentration have been reported to be a marker for CVD and results from the present study also lead to the same conclusion. Comparison between serum Lp(a) concentration and the molecular size of the Lp(a) molecule present are reported to have an inverse relation. Hence it is expected that high serum Lp(a) individuals carry low molecular weight Lp(a) moieties in their circulation. But the present study findings show that patients with CVD have predominance of high molecular weight Lp(a) isoforms compared to those present in normal control individuals. Keep in view the above finding and the fact that diabetic CVD patients have 50% higher load of Lp(a)-IC, it can be concluded that high molecular weight Lp(a) present in CVD patients are easily prone to desialylation and immune complex formation. Such immune complexes with large size easily form lattice structures which can get bound to cells and endothelial linings as is shown by hemagglutination experiments. A similar mechanism whereby monocytes can adhere to the immune complexes and act as a vehicle to transport Lp(a)-IC to subendothelial layer.

16. Summary sheet of not more than 2 pages under following heads :

Title: Desialylation-driven uptake of lipoprotein(a) to endothelial cells and monocytes / macrophages in diabetic cardiovascular patients: Is immune complex with natural antibodies a vehicle?

Introduction: Presence of several times more Lp(a) than LDL in atherosclerotic plaques while the reverse is the situation in circulation warrant investigation into the role of Lp(a)-ICs in atherosclerosis. Another fact is that Lp(a) as a potential causal, genetic and independent risk factor for CVD. Diabetes is a chronic disease and organelle dysfunction including that of lysosomes has been observed as hallmark of diabetes leading to release of enzyme neuraminidase which causes desialylation of membrane and secreted glycoproteins. Since glycoconjugate desialylation is systemic and extensive in the case of diabetes, endothelial cells may also get desialylated on their surface glycans, thus offering extensive binding sites for Lp(a) IC in diabetic patients. This offers a very clear route for (a) Lp(a) entry into subendothelial layers and (b) vascular immune inflammation. Both these events can act synergistically towards atherosclerosis.

The major question for which we seek answer through this project is: Do diabetic patients produce abnormal Lp(a) IC in their circulation? If the answer is yes, it offers a way for inhibiting the IC formation by introducing into circulation, high specificity synthetic or natural oligosaccharides or

polypeptides to inhibit the concerned antibodies so that formation of Lp(a) IC characteristic of diabetes are prevented.

Rationale:

Overall mortality due to CVD and diabetes is on an increase globally but much faster in developing countries like India. Although the state of Kerala stands highest in literacy rate, the control rates of diabetes are poor. Among the diagnosed diabetics only 68% control the disease by medications, 17% do not take any treatment where 15% were on diet alone. CVD accompanies uncontrolled diabetes resulting in an ever increasing burden of CVD patients in the state. A method to control the complications of CVD caused by Lp(a) immune complexes formed in diabetes using glycopeptide therapy is a distinct therapeutic possibility in this scenario. This study aims at the relevance of Lp(a) immune complex in CVD patients as well as diabetic CVD patients.

Objectives:

1. Isolation and characterization of plasma Lp(a) immune complex in order to assess desialylation status of Lp(a) in CVD as well as diabetic CVD patients and comparison to normal healthy individuals

2. Elucidation of relevance of molecular size and concentration of Lp(a) in CVD using newer protocols for determining Lp(a) size and assay of plasma levels developed in this lab.

3. Assay of binding in vitro of desialylated Lp(a) IC to human endothelial cells/ monocytes / macrophages/ human red cell after exposing the latter to diabetic levels of neuraminidase

Methodology:

10ml of fasting blood sample from three categories of volunteers was collected in vacutainer tubes for serum collection.(a) Normal individuals as healthy control volunteers (b) CVD patients with diabetes (c) CVD patients with no history of diabetes. Following experimental methods were adopted on these three types of samples

1. Serum Lp(a) Assay
2. Assay of the desialylation status of Lp(a) in immune complex Lp(a)-IC in serum
3. Ratio of mobility of LDL to Lp(a) in TBE electrophoresis
4. Antibody distribution in serum Lp(a) immune complex
5. Haemagglutination by Lp(a) IC

Results:

1. Mean serum Lp(a) value in normal control volunteers was found to be 18.8 mg/dL whereas in cardiovascular disease (CVD) affected individuals , the mean serum Lp(a) value is found to be 29 mg/dL. This shows that there is a 55% increased serum Lp(a) concentration in CVD patients as compared to normal individuals. Whereas diabetic and non-diabetic CVD affected patients are not much different as far as their serum Lp(a) value is concerned (diabetic: 28.2 mg /dL and non-diabetic: 30 mg/dL)
2. Diabetic individuals carry **88%** higher levels of desialylated Lp(a) in serum compared to normal control individual whereas non-diabetic individuals carry only **8.97%** higher levels of desialylated Lp(a) in serum compared to normal control individual.

3. Compared to control individuals, CVD patients have an average **30%** higher load of Lp(a)-IC in circulation and it was found that CVD patients with **diabetes** have **50%** whereas **non-diabetic CVD** have **11.7%** higher load of Lp(a)-IC. IgG is the dominant immunoglobulin present in Lp(a) –IC from any category of samples.
4. Lp(a)-IC from control individuals showed no red cell agglutination. At the same time immune complexed Lp(a) from diabetic-CVD patients agglutinated desialylated red cells up to a dilution of 1: 16 and Lp(a)-IC from non-diabetic patients agglutinated desialylated RBC upto a dilution of only 1:2
5. The TBE electrophoresis clearly shows Lp(a) and LDL bands (proven by ELISA) and the ratio of mobility of LDL to Lp(a) [LDL : Lp(a)] is taken in the experiments to determine the molecular size of Lp(a). It is observed that in control volunteers the ratio is 1:8 whereas in CVD patients it is 2:8 showing a 57% higher molecular sized Lp(a) in CVD patients.
6. Lp(a) is purely heritable and its serum levels are largely determined by apo(a) gene expression. It is an observation from among the samples processed that in normal control samples only 7.4% had more than one Lp(a) band whereas 33.3% of CVD samples had more than one band.

Translational potential:

Lp(a) has been a proven genetic factor for cardiovascular deficits and along with that diabetes makes the situation worse. Results from this work explains this fully. This study involving Lp(a) IC and its desialylation status in cardiovascular patients with and without diabetes in comparison with healthy individuals throws light on the role of diabetes in cardiovascular condition. The ultimate culprit is Lp(a) immune complex that creates the whole scenario leading to inflammatory reactions. Since Lp(a) is a genetically determined product with variable functions in the body, lowering its concentration in circulation does not seem to be effective. So ways to lower its harmful effects should be unveiled which includes possibility of preventing IC formation and atherosclerosis in diabetics using biological glycopeptides as inhibitors of Lp(a) IC formation. Further, the Lp(a) IC involving anti-carbohydrate antibodies may be utilized in studying the desialylation status of monocytes and endothelial cells in such individuals. study involving Lp(a) IC and their desialylation status in cardiovascular patients with and without diabetes in comparison with healthy individuals may throw light on the role of diabetes in cardiovascular inflammation. Results may offer possibility of preventing IC formation and atherosclerosis in diabetics using biological glycopeptides as inhibitors of Lp(a) IC formation.

17. Contributions made towards increasing the state of knowledge in the subject :

1. Serum Lp(a) assay was done using jacalin based assay protocol and the following observations were made. Mean serum Lp(a) value in normal control volunteers was found to be 18.8 mg/dL whereas in cardiovascular disease (CVD) affected individuals , the mean serum Lp(a) value is found to be 29 mg/dL. This shows that there is a 55% increased serum Lp(a) concentration in CVD patients as compared to normal individuals. Whereas diabetic and non-diabetic CVD affected patients are not much different as far as their serum Lp(a) value is concerned (diabetic: 28.2 mg /dL and non-diabetic: 30 mg/dL)
2. Desialylated Lp(a) content in the Lp(a)-immunecomplex in circulation is ascertained using the lectin peanut agglutinin as the tool to measure desialylation and the ratio of immune complexed Lp(a) to circulating Lp(a) is determined in all the three categories of individuals.

Diabetic individuals carry **88%** higher levels of desialylated Lp(a) in serum compared to normal control individual whereas non-diabetic individuals carry only **8.97%** higher levels of desialylated Lp(a) in serum compared to normal control individual.

3. Assay of serum Lp(a)-IC prepared by poly ethylene glycol precipitation was done by ELISA . Compared to control individuals, CVD patients have an average **30%** higher load of Lp(a)-IC in circulation and it was found that CVD patients with **diabetes** have **50%** whereas **non-diabetic CVD** have **11.7%** higher load of Lp(a)-IC. IgG is the dominant immunoglobulin present in Lp(a) –IC from any category of samples.
4. To ascertain free binding sites on Lp(a)-IC, haemagglutination assay was performed. Lp(a)-IC from control individuals showed no red cell agglutination. At the same time immune complexed Lp(a) from diabetic-CVD patients agglutinated desialylated red cells up to a dilution of 1: 16 and Lp(a)-IC from non-diabetic patients agglutinated desialylated RBC upto a dilution of only 1:2
5. The study also evaluated whether there is any molecular size variation of Lp(a) in normal and CVD affected individuals. It is already reported that most of the Lp(a) in circulation is in an adduct form with LDL and the quantity of LDL bound to Lp(a) is proportional to the molecular size of Lp(a). Hence in jacalin precipitated Lp(a) preparations LDL is an indispensable component. The TBE electrophoresis clearly shows Lp(a) and LDL bands (proven by ELISA) and the ratio of mobility of LDL to Lp(a) [LDL : Lp(a)] is taken in the experiments to determine the molecular size of Lp(a). It is observed that in control volunteers the ratio is 1:8 whereas in CVD patients it is 2:8 showing a 57% higher molecular sized Lp(a) in CVD patients.
6. Lp(a) is purely heritable and its serum levels are largely determined by apo(a) gene expression. It is an observation from among the samples processed that in normal control samples only 7.4% had more than one Lp(a) band whereas 33.3% of CVD samples had more than one band.

18. Conclusions summarising the achievements and indication of scope for future work :

Diabetes is one clinical condition in which significant increase in neuraminidase enzyme in circulation and consequent desialylation of plasma and tissue glycoconjugates takes place. A surge in neuraminidase in plasma can therefore result in circulating Lp(a) IC with free binding sites. These ICs can get lodged to the desialylated cells or surfaces and pave way for inflammatory vascular diseases. The reported presence of more apo(a) than apoB in atherosclerotic plaques eventhough LDL is many times more in circulation than Lp(a), highlights the role of Lp(a) in vascular pathology. It may also provide an explanation for the mechanism of entry of Lp(a) in the sub-endothelial space and then to plaque since no apo(a) receptor is reported on the endothelial surface.

Considering the **concentration of Lp(a) and its immune complex** in serum, patients with cardio vascular disease have 55% higher serum. Lp(a) and 30% higher Lp(a)-immune complex when compared to normal control volunteers with no evident cardiovascular deficit proving that Lp(a) has a dominant role in atherogenesis.

Desialylation occurring in a diabetic patient was taken as a scale to measure the extent to which diabetes can be a contributing factor for vascular diseases. Since desialylation of

Lp(a) paves way for its immune complex formation the **content of desialylated Lp(a) in Lp(a) immune complex** of diabetic CVD patients was compared to non-diabetic CVD patients. Diabetic individuals carry **88%** higher levels of desialylated Lp(a) in serum compared to normal control individual whereas non-diabetic individuals carry only **8.97%** higher levels of desialylated Lp(a) in serum compared to normal control individual.

The molecular weight of Lp(a) is another factor expected to contributes to vascular diseases. Larger Lp(a) moieties have more number of antibody binding epitopes and hence higher chances of immune complex formation. Results regarding the molecular weight of Lp(a) in all three categories of samples revealed that in CVD patients the Lp(a) moieties present are around 57% higher in molecular size. **Heamagglutination** capability of Lp(a) immune complex from all three categories of samples was compared to assess free binding sites on these complexes and it was observed that Lp(a)-IC from normal volunteers showed hardly any red cell agglutination whereas immune complexed Lp(a) from diabetic-CVD patients was four times better in heamagglutination than Lp(a)-IC from non-diabetic patients. Experiments showed that IgG is the major antibody forming immune complex with Lp(a).

Lp(a) has been a proven genetic factor for cardiovascular deficits and along with that diabetes makes the situation worse. Results from this work explains this fully. This study involving Lp(a) IC and its desialylation status in cardiovascular patients with and without diabetes in comparison with healthy individuals throws light on the role of diabetes in cardiovascular condition. The ultimate culprit is Lp(a) immune complex that creates the whole scenario leading to inflammatory reactions. Since Lp(a) is a genetically determined product with variable functions in the body, lowering its concentration in circulation does not seem to be effective. So ways to lower its harmful effects should be unveiled which includes possibility of preventing IC formation and atherosclerosis in diabetics using biological glycopeptides as inhibitors of Lp(a) IC formation. Further, the Lp(a) IC involving anti-carbohydrate antibodies may be utilized in studying the desialylation status of monocytes and endothelial cells in such individuals.

19. Science and Technology benefits accrued

a) **List of research publications with complete details : NIL**

b) **Manpower trained on the project**:

1) Research scientists or research fellows: **NIL**
2) No. Of PhDs produced**: NIL**
3) Other technical persons trained: **Project Assistant (2 Nos.)**

c) **Patents taken: NIL**

d) **Products developed: NIL**

20. Abstract

a) Background

Presence of more Lp(a) than LDL in atherosclerotic plaques while the reverse is the situation in circulation warrant investigation into the role of Lp(a)-ICs in atherosclerosis. Lp(a) is a potential causal, genetic and independent risk factor for CVD. In chronic diabetes organelle dysfunction including that of lysosomes leads to release of neuraminidase which causes desialylation of membrane and secreted glycoproteins. Since glycoconjugate desialylation is systemic and extensive in the case of diabetes, endothelial cell surface glycans may also get desialylated on their surface glycans, thus offering extensive binding sites for Lp(a) IC. This offers a very clear route for (a) Lp(a) entry into subendothelial layers and (b) vascular immune inflammation. Both these

events can act synergistically towards atherosclerosis.

b) Materials

Polystyrene wells, jacalin, Tween-20, standard Lp(a), anti-apo(a), anti-human IgA, IgG, IgM antibody, Orthophenylene diamine, Citrate-phosphate buffer, Hydrogen peroxide, Sulfuric acid, Poly ethylene Glycol, Urea, KBr, Peanut Agglutinin , Tris, Boric acid, Acrylamide, Horse Radish Peroxidase Neuraminidase enzyme, O-group human Red Cells

c) Results: In comparison to normal individuals CVD patients have increased serum Lp(a) concentration and diabetic individuals carry higher levels of desialylated Lp(a). Study to determine the molecular size of Lp(a) shows that patients with CVD have higher molecular sized Lp(a) as well as a higher load of Lp(a) IC more so in CVD patients with diabetes. IgG is the dominant immunoglobulin in Lp(a) IC. Lp(a)-IC from control individuals showed no red cell agglutination whereas those from diabetic-CVD patients agglutinated desialylated red cells more thanthat from non-diabetic patients Lp(a) is purely heritable and its serum levels are largely determined by apo(a) gene expression. It is an observation from among the samples processed that in normal control samples only 7.4% had more than one Lp(a) band whereas 33.3% of CVD samples had more than one band.

d) Conclusion: To conclude, serum Lp(a) and Lp(a)-immune complexes rich in IgG are significantly high in CVD patients. Diabetes definitely contributes to cardiovascular pathology because among CVD patients, diabetes affected CVD volunteers had high levels desialylated Lp(a) in their immune complexes. Free binding sites on the immune complex from diabetic patients was also significantly high as shown by hemagglutination, which provide them with best anchoring capacity to endothelial surfaces that create an environment suitable for atherogenesis. In normal control samples only 7.4% had more than one Lp(a) band whereas 33.3% of CVD samples had more than one band. Such an observation is not reported yet and clinical correlation needs further extensive research. Hence these findings explains the possibility of Lp(a) immune complex acting as a vehicle for Lp(a) transportation to sub-endothelial space.

21. Procurement / usage of equipments:

a) **Details of equipments**

Sl. No.	Name of equipment	Make & Model	Cost (INR)	Date of Installation	Utilisation	Remarks regarding maintenance breakdown
1	Printer	EPSON LQ310 DOT MATRIX	11,000	30 Mar, 2019	100%	NIL
2	pH meter	EUTECH CYBERSCAN PH510	33,900	19 Nov, 2018	100%	NIL
3	Refrigerator	SAMSUNG 345 LTR 4 STAR	21,000	10 Dec, 2018	100%	NIL
4	Laboratory centrifuge	EPPENDORF 5702	1,96,200	22 Jun, 2019	100%	NIL
5	Micropipettes	EPPENDORF PIPETTES	1,38,538	18 Dec, 2018	100%	NIL

b) Suggestions for disposal of equipments:

The research laboratory where the present study was carried out is a full fledged research space with sophisticated equipments and is totally dedicated for the cause. The

equipments purchased under this project are the basic minimal requirements of a laboratory and are taken into Biochemistry Department stock. These equipments will be continuously in use in the laboratory and when become out of use or faulty will be condemned through the institute condemnation protocol.

Name of PI: Dr. Geetha M
Date: 28-11-2023