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Coagulation Factor 1, Oxidative Stress Marker And Antioxidant Level In Predicting The Outcome Of Diabetic Foot Ulcer

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ABSTRACT:

INTRODUCTION:Diabetic Foot Ulcer is the complication of diabetes mellitus that impacts heavily on the quality of life. Diabetic foot ulcer is considered as the most important cause of lower extremity amputation.The pathogenesis of diabetic foot ulcer is multifactorial and complex.It is associated with microvascular and macrovascular complications. Oxidative stress marker plasma Malondialdehyde(MDA) plays an important role in the pathogenesis of diabetic foot ulcer. Coagulation factor 1 Fibrinogen plays an important role as the inflammatory marker of vascular changes and endothelial dysfunction.

AIM & OBJECTIVE: The aim of the study is to assess and correlate the levels of vitamin C , Plasma Fibrinogen &Plasma MDA with prognosis in diabetic foot ulcer patients Grade 1&2 and along with healthy controls.

MATERIALS &METHODS: This study includes 100 Diabetic patients (30 without complication, 35 diabetic foot ulcer Grade1&35 with grade 2) and 30 age matched healthy controls.

After getting informed consent from the patient , 5ml of venous blood sample was collected in EDTA coated tube. Plasma MDA was assayed by Esterbauer and Steinberg method. Plasma Fibrinogen was measured in mispa coagulometer. Plasma Vitamin C was measured by Roe & Kuether method . HbA1C was calculated for all the subjects by Draca fully automated analyser. haemoglobin was measured in Sahli's hemoglobinometer

RESULTS: Oxidative stress marker and Plasma Fibrinogen levels were markedly increased in Grade 2 ulcer patients followed by Grade 1 patients and diabetes without complications. Plasma levels of vitamin C significantly reduced in grade 1 and 2 diabetic foot ulcer patients .

CONCLUSION: Poor prognosis of diabetic foot ulcer can be explained by the increased levels of Oxidative stress and Coagulation Factor1 and decreased levels of vitamin C irrespective of Glycemic control.

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1. INTRODUCTION

Diabetes mellitus is a group of metabolic disorder of carbohydrate metabolism in which glucose is underused producing hyperglycemia. The vascular complications of type-2-diabetes- mellitus (T2DM) can be caused by either microangiopathy or macroangiopathy. Diabetic foot ulcer is the leading cause of lower extremity amputation and is generally known to have poor prognosis. Diabetic foot is a serious diabetic complication that refers to the destruction of the skin and deep tissues (including muscle and bone) distal to the ankle joint, often combined with arterial occlusion and infection of the lower extremity. The pathogenesis of diabetic foot ulcer is multifactorial. Fibrinogen is a recognized marker in peripheral vascular disease; increasing levels predict an increased mortality and risk of amputation. Plasma fibrinogen is an inflammatory marker that has important role in the pathogenesis of inflammation, atherosclerosis, thrombogenesis, and development of vascular complications in T2DM patients[1][2]. Diabetic foot ulcer is one of the most common causes of non traumatic lower extremity amputation and also the most frequent reason for hospitalisation in T2DM patients . In T2DM patients small injuries from shoes and trivial trauma are not perceived owing to neuropathy and these injuries subsequently result in non healing ulcer . The etiopathogenesis of diabetic foot ulcer includes various causes such as hyperglycemia, neuropathy, endothelial dysfunction and oxidative stress, deviant metabolic state in T2DM involves chronic hyperglycemia , dyslipedemia and insulin resistance. This aberrant metabolic condition affects the function of endothelial cell, smooth muscle cells and cells associated with inflammation. The endothelial cells of the vascular compartment secrete various bioactive substances, which regulate vascular function and inflammation. Oxidative stress (MDA) is considered important in the pathogenesis of chronic wounds[3]. In T2DM, the secretion of vascular relaxing factor is impaired and the existing hyperglycemia induces the production of reactive oxygen species (ROS). In T2DM insulin resistance causes excessive release of fatty acids from the adipose tissue, which results in concomitant inhibition of phosphatidylinositol-3 kinase (IP-3) pathway and activation of protein kinase C, thus increasing the production of ROS[4]. Besides an increased production of ROS, the diabetic state induces the generation of many vasoactive substances and vasoconstrictors which lead to vascular smooth muscle hypertrophy. Vitamin C (ascorbic acid) is a powerful anti oxidant reducing the risk of cancer. Ascorbic acid is necessary for post translational hydroxylation of proline and lysine residues . Hydroxyl proline and hydroxyl lysine are essential for the formation of cross links in the collagen which gives the tensile strength to the fibres . In this way , vitamin C is necessary for wound healing process .Hence the deficiency of vitamin C causes delayed wound healing .

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Aim & Obective:

The aim of the study is to assess and correlate the levels of Plasma Fibrinogen , Plasma vitamin C (ascorbic acid) and Plasma MDA with prognosis in diabetic foot ulcer patients Grade 1&2 and along with healthy controls

2. MATERIAL AND METHODS

Place of study : Department of Biochemistry in association with Department of General surgery, Vinayaka mission's Medical College and Hospital, Karaikal .

Duration of study : June 2021 – June 2022

Inclusion criteria : The study included 100 diabetic patients (30 diabetic without complication, 35 diabetic foot ulcer grade 1 and 35 diabetic foot ulcer grade 2 patients) . 30 age matched healthy controls. the diabetic foot ulcer patients were classified as per wagner's ulcer classification. Diabetic foot ulcer grade 1 patients includes superficial ulcers not involving tendon, bone or capsule . Diabetic foot ulcer grade 2 patients includes deep ulcer exposing bone or ligament.

Exclusion criteria :

Diabetic foot ulcer – grade 3 ,4 and 5 . Patient already undergone amputation .Chronic wounds caused by other etiologies than diabetes , Diabetic ketoacidosis,cardiac patients , diabetic retinopathy,diabetic nephropathy and atherosclerosis .

After getting informed consent from the patient , 5ml of venous blood sample was collected in EDTA coated tube. Plasma MDA was assayed by Esterbauer and Steinberg method. Plasma Fibrinogen was measured in mispa coagulometer. Plasma Vitamin C was measured by Roe & Kuether method . HbA1C was calculated for all the subjects by Draca fully automated analyser. haemoglobin was measured in Sahli's hemoglobinometer.

3. RESULTS

Table 1 shows number of patients in each group and number of healthy controls and site of ulcer . Table 2 shows the biochemical parameters like haemoglobin,HbA1C, MDA, Fibrinogen and vitamin c. The level of hemoglobin level was lowered in diabetic foot ulcer grade 1&2 patients when compared to diabetes without complication and healthy controls. There was a significant increase in the levels of plasma malondialdehyde which is an oxidative stress marker and plasma fibrinogen which is an inflammatory marker in diabetic foot ulcer grade 2 patients followed by diabetic foot ulcer grade 1 patients followed by diabetes without complications and healthy individuals[6] . Plasma vitamin C level was drastically reduced in diabetic foot ulcer patients than control.

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Table 1 :

Total no. Of patients	130
DIABETIC FOOT ULCER grade 2	35
DIABETIC FOOT ULCER grade1	35
Diabetes without complications	30
Healthy controls	30
Age	45±10
Forefoot ulcer	40
Midfoot ulcer	20
Hindfoot ulcer	10

Table 2 :

Parameters	Diabetes without complications	DIABETIC FOOT ULCER grade1	DIABETIC FOOT ULCER grade 2	Controls
Haemoglobin (g/dl)	10.4 ± 1.2	9.5 ± 0.6	9.1 ± 0.4	11.2 ± 1.3
HbA1c (%)	7.1 ± 0.2	8.2 ± 0.8	9.8 ± 0.3	5.2 ± 0.5
MDA (µmol/L)	4.6 ± 0.8	6.2 ± 0.4	6.8 ± 1.2	3.8 ± 1.4
FIBRINOGEN (mg/dl)	298.56 ± 68.25	356.34 ± 45.23	423.45 ± 66.72	235.23 ± 35.91
VITAMIN C (mg/dl)	0.55 ± 0.21	0.48 ± 0.28	0.35 ± 0.2	1.1 ± 0.25

4. DISCUSSION

Diabetic foot ulcer is due to microangiopathy which affect microcirculation. Increased HbA1c level decrease the oxygen dissociation thereby oxygen utilisation is decreased. Plantar aspect of the foot is the commonest site followed by leg, upper limb , back ,scrotum and perineum.

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Wagner's grading of ulcer
Grade 0- pre ulcerative / healed ulcer
Grade 1 – superficial ulcer
Grade 2 – ulcer deeper to subcutaneous tissue exposing soft tissue or bone
Grade 3- abscess formation / osteomyelitis
Grade 4 – gangrene of the part of tissue / limb/foot
Grade 5 – gangrene of entire foot

This study analysis the association of oxidative stress, plasma vitamin c, plasma fibrinogen and prognosis of diabetic foot ulcer. Oxidative stress is known to be associated in the development of insulin resistance, β cell dysfunction and impaired tolerance to glucose in T2DM patients . Oxidative stress also causes the long term complications of DM such as microvascular and macrovascular complications. Lipid peroxidation occurs due to imbalance between free radicals and antioxidant production. Excessive formation of free radicals and increased glycation of proteins results in oxidative stress[8]. The glycated protein products are the source of free radicals. The highest plasma MDA level in severe diabetic foot ulcer patients can be explained by the increased production of free radicals. Free radicals interact in arachidonic acid metabolism. The lipid peroxide formed stimulates the cyclo oxygenase and prostaglandin and thromboxane synthesis leading to increased platelets aggregation, causing vascular complications and tissue damage in severe diabetic foot ulcer patients. In vitro studies demonstrated an increase in oxidative stress in cells when exposed to a hyperglycemic environment . A positive correlation of diabetic foot ulcer with oxidative stress is identified in our study[11].

Coagulation factor one is fibrinogen. It is an inflammatory marker and an acute phase reactant.It is also an important determinant of blood viscosity and platelet aggregation and a risk factor for vascular events. It plays an major role in pathogenesis of inflammation, thrombogenesis and development of micro vascular and macro vascular complications in type-2 diabetes mellitus patients[7]. In this present study, plasma fibrinogen level was remarkably increased in all diabetic foot ulcer grade 2 patients compared to diabetic foot ulcer grade 1 followed by diabetes without complications and healthy controls.When fibrinogen level increase in blood , it would cause increased blood viscosity thereby it would increase fibrin clot thickening which deposit in damaged endothelium producing atherogenic thrombi . When the metabolic response is poor to these cascade , there would be chronic hypersecretion which induce chronic plaque aggregation . The conversion of fibrinogen to fibrin occurs by cleaving of Arg-gly peptide of fibrinogen[9]. Fibrinogen synthesis is regulated by a feedback mechanism by thrombin activation .Molecular weight of fibrinogen is 340,000 D and it is synthesised by liver . Normal level of fibrinogen in blood is 200- 400 mg/dl. They align themselves lengthwise , aggregate and form the clot[10] . In diabetics free radicals also induce thrombin formation. Thus oxidative stress is a link between increased fibrinogen levels in diabetics. The higher level of plasma fibrinogen is due to increase in the synthesis and decreased clearance of fibrinogen. Hence this study shows a positive correlation of increased plasma fibrinogen with diabetic

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foot ulcer [5]. Similar study supporting our study was conducted by Naga RV in which plasma fibrinogen level was significantly higher in all diabetic foot ulcer grade 2 patients compared to diabetic foot ulcer grade 1 [1]. Other data also revealed a significant association between fibrinogen and diabetic foot ulcer disease severity as reflected by amputation.

vitamin C is an antioxidant that helps to protect our cells against the effects of free radicals. Vitamin C is a water soluble vitamin which is also known as ascorbic acid. Humans cannot synthesise ascorbic acid because of the lack of enyme L-gulono lactone oxidase. The vitamin therefore, should be supplied in the diet. Vitamin c is necessary for the growth, development and repair of all body tissues. It's involved in many body functions, including formation of collagen, absorption of iron, the proper functioning of the immune system, wound healing, and the maintenance of cartilage, bones, and teeth[12]. Vitamin c is essential for wound healing and hence deficiency of vitamin c in diabetic foot ulcer patients causes delayed wound healing. Also the decreased level of antioxidant vitamin C in diabetic foot ulcer leads to increased oxidative stress in these patients resulting in vicious cycle. This study shows an association between increased oxidative stress level and decreased vitamin c level in diabetic ulcer grade 2 patients[13]. Patients with grade 1 and 2 ulcer were anemic when compared with diabetes without complications and controls.

Conclusion

The present study has demonstrated that diabetic foot ulcer grade 2 patients have very high levels of oxidative stress followed by diabetic foot ulcer grade 1 and followed by diabetes without complications and healthy controls. This study also shows the decreased levels of vitamin c in diabetic foot ulcer grade 2 and 1 patients when compared with healthy controls. Therefore supplementation of vitamin c helps in reducing further progression of the disease. Plasma fibrinogen levels are also significantly elevated in patients with diabetic foot ulcer grade 2 followed by diabetic foot ulcer grade 1 and followed by diabetes without complications and healthy controls. Hence Fibrinogen and MDA levels can be used to predict the severity of diabetic foot ulcer. Thus, better glycemic control to reduce hyperglycemia induced oxidative stress and also reduction in fibrinogen levels in diabetic foot ulcer patients may be helpful in the management of diabetic foot ulcer. To conclude coagulation factor 1 otherwise called as fibrinogen could be considered as an inexpensive,easily applicable screening test for a rapid assessment of diabetic foot ulcer severity to do timely intervention.

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