ISSN: 2279 - 0594

Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
Volume 4, Issue 3, May-June, 2015, 56-65

Research Article

STUDY ON MATRIX METALLOPROTEINASES IN DIABETIC FOOT ULCER DISEASE

Julie Susan Sam* and Baskaran Krishnan

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar, Tamil Nadu, India.

Received 18 May 2015; Accepted 02 June 2015

ABSTRACT

The aim of this study is to compare the level of matrix metalloproteinases in diabetic and non-diabetic foot ulcer disease patient's plasma and tissue sample. **Design**: Cross-sectional study. **Patients**: We studied a group of adults with T2DM (n=10) and another group of adults without T2DM (n=6). **Measurements**: MMPs in plasma and tissue samples were detected by gelatin zymography. **Results**: The plasma and tissue concentrations of MMP-9 and MMP-2 and non-enzymatic antioxidants were found to be increased in diabetic foot ulcer patients compared to non-diabetic foot ulcer patients. **Conclusion**: The elevated levels of MMP-9 and -2 in chronic ulcer subjects, delays wound healing process by degrading the extracellular matrix materials in an uncontrolled way. This study provides evidence for the role of MMPs 2 and 9 in diabetic foot ulcer disease.

Key words: Matrix metalloproteinases, gelatin zymography, diabetic foot ulcer

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism result from defects in insulin secretion, insulin action, or both. The complications of diabetes mellitus include retinopathy, nephropathy, and neuropathy (both peripheral and autonomic). Diabetic neuropathy and lower extremity vascular disease combine to make diabetes the leading cause of non-traumatic lower extremity amputations. Chronic elevation of blood glucose level leads to damage of blood vessels (angiopathy). In diabetes, the resulting problems are grouped under "microvascular disease" (due to damage to small blood vessels) and "macrovascular disease" (due to damage to the arteries). Diabetic foot ulcer disease has become a threat among diabetic patients as they are subjected to amputations which cause considerable morbidity and mortality. The amputation rate due to diabetic foot ulcer is increasing every year.

An estimated 15% of patients with diabetes will develop a lower extremity ulcer during the course of their disease. Several population-based studies indicate a 0.5% to 3% annual cumulative incidence of diabetic foot ulcers [35, 42, 22, 34]. Neuropathy, deformity, high plantar pressure, poor glucose control, duration of diabetes, and male gender are all contributory factors for foot ulceration [44, 14, 13, 6]. National hospital discharge data indicate that the average hospital length of stay (LOS) for diabetic patients with ulcer diagnoses was 59% longer than for diabetic patients without ulcers. While 7% to 20% of patients with foot ulcers will subsequently require an amputation, foot ulceration is the precursor to approximately 85% of lower extremity of amputations in persons with diabetes [15]. Patients with diabetes are 25 times more likely to lose a leg than those without the condition, and up to 70% of all leg amputations occur in people with diabetes. The result is that a leg is lost to diabetes every 30 seconds somewhere in the world [4]. In diabetes, nerve damage results from interacting metabolic abnormalities, worsened by disease of the vasa nervorum. The damage affects peripheral sensation, innervations of the small muscles of the foot, and fine vasomotor control of the pedal circulation. In sensory neuropathy, loss of protective sensation leads to lack of awareness of incipient or actual ulceration. Motor neuropathy affects the muscles required for normal foot movement, altering the distribution of forces during walking and causing reactive thickening of skin (callus) at sites of abnormal load. Ischaemic necrosis of tissues beneath the callus leads to breakdown of skin and subcutaneous tissue, resulting in a neuropathic ulcer with a punched-out appearance. Arteriolar-venular shunting causes dysfunction of the microcirculation with reduced distribution of blood to areas of need. Thus, tissue ischaemia can occur in a foot with palpable pedal pulses. Vasomotor (autonomic) neuropathy affects the peripheral nerve function, which controls the distribution of blood through arteriolar vessels. As with the micro vascular complications of diabetes, there is a clear

**Corresponding author: Julie Susan Sam | E-mail: juliesusansam@gmail.com*

relationship between glycemic control and measures of neuropathy. The DCCT (Diabetes control and complications trial) intensively controlled subjects had a 60% reduction in neuropathy.

Foot tissues can become ischaemic because of macrovascular disease (atherosclerosis), notably in the calf with relative sparing of proximal vessels and those in the foot. Ischaemia also results from micro vascular disease— both structural (thickened basement membrane, capillary wall fragility, and thrombosis) and functional (vasomotor neuropathy with defective microcirculation and abnormal endothelial function). Protective sweating is lost and the skin of the ischaemic foot is red, dry, thin with dystrophic nails, and susceptible to the pressure from a shoe or even an adjacent toe [19].
In diabetic patients the cellular and the inflammatory pathways involved in wound healing are affected. In diabetes hyperglycaemia particularly through hyperosmotic effects influence neutrophils and fibroblasts breaking the healing cascade [24]. The faulty healing response is additionally affected due to the wound hypoxia caused by the microvascular and macrovascular conditions of the diabetic patients [20]. The microvascular conditions lead to injury in small blood vessels often leading to vasoconstriction. As the condition progresses the vascular abnormalities affect the membranes of the blood vessels leading to macrovascular conditions mainly peripheral vascular disease affecting the leg artery. Neuropathy predisposes the foot to infection and angiopathy influences the outcome [10,3]. Autonomic neuropathy causes dry skin and predisposes the skin to cracking. A foot with sensory neuropathy tends to suffer repeated injury, thus disrupting the skin integrity and providing a route for microbial invasion leading to an unhealed wound which further develops into a chronic ulcer [5]. Motor neuropathy causes atrophy of the intrinsic foot muscles, altering the foot architecture leading to osteomyelitis. Due to the higher glucose levels and free fatty acid metabolism there is an oxidative stress in the wound often referred to as ischemic wound which renders a perfect environment for the anaerobes. Patients with these additional risk factors such as ischemia, neuropathy and peripheral vascular disease often have unsuccessful inflammatory responses and hence have weakened immune system. One of the peculiar characteristics often seen in the chronic wound within an immuno-compromised diabetic patient is replicating micro-organisms. Additionally the excess sugar lowers the resistance to infections which further leads to a gangrenous ulcer with lower limb amputations [17]. About 60% of the amputations are as a result of infection of the diabetic foot ulcer making infection the most important pathway of diabetic foot. Infection, however, is never the sole cause of diabetic foot and other risk factors include increasing age, sex, race, smoking habits, duration of diabetes and glycated haemoglobin (HbA1C) [31]. The history of previous hospitalisations for the same wound, duration of the wound, duration of hospital stay, osteomyelitis, previous antibiotic therapy and non-healing gangrenous foot ulcer can affect the diabetic foot ulcer and its prognosis [46]. Thus multiple factors contribute to the aetiopathogenesis of diabetic foot infections [37].

MMPs are a family of zinc endopeptidases capable of degrading all the components of the extracellular matrix. They are key players in every phase of the healing process: they eliminate damaged protein, destroy the provisional extracellular matrix, facilitate migration to the centre of the wound, remodel the granulation tissue, probably control angiogenesis and also regulate the activity of some growth factors [28]. Based on domain organization and substrate preference, MMPs are grouped into collagenases, gelatinases, stromelysins, matrilysins, membrane-type (MT)-MMPs and others.
Matrix metalloproteinases (MMPs) have been shown to play significant roles in a number of physiological as well as pathological processes. Matrix metalloproteinases (MMPs) have been shown to play significant roles in a number of physiological as well as pathological processes. Best known to degrade components of the extracellular matrix, MMPs have recently been discovered to also target a growing list of proteins apart from these, both inside and outside the cell. MMPs have also been traditionally thought of as enzymes involved in chronic processes such as angiogenesis, remodelling and atherosclerosis on a days-week time-scale. However they are now understood to also act acutely in response to oxidative stress on a minute time-scale on non-extracellular matrix substrates. Two prominent MMP family members are MMP-2 and -9. The MMP-9 is secreted as a 92 kDa zymogen. Cleavage of Pro-MMP-9 results in the active enzyme, having a molecular weight of approximately 82 kDa. Pro-MMP-2 has a molecular weight of 72 kDa. After cleavage, active MMP-2 has a molecular weight of 67 kDa.

MMPs are finely regulated and a successful healing process is dependent on a rigorous spatial and temporal pattern of expression. Whereas MMP levels decrease through the normal wound-healing process, chronic wounds contain a significantly higher level of proteases and pro-inflammatory cytokines (tumour necrosis factor-α and interleukin-1), as well as lower levels of growth factors. In particular, collagenase and gelatinase activity is

increased in chronic wounds, while the TIMP-1 level is reduced compared to acute wounds [28].

Poor glucose control contributes to the altered healing in diabetic wounds. Prevention of hyperglycemia improves healing in animals made diabetic by destruction of the islets of Langerhans [18].

MATERIALS AND METHODS:

This study protocol was approved by the Institutional Human Ethics Comittee (IHEC) and all the patients were given written informed consent for all procedures. Patients with diabetic foot ulcer visiting the Department of Surgery, Rajah Muthiah Medical College, Annamalai Nagar were included for the present study. A detailed history of duration of diabetes, type of therapy and triggering factor for foot ulcer were noted. Blood samples were collected on the day of admission and after two weeks (antibiotics and drugs for local application) of standard therapy. Samples were collected from patients of either sex with age 25 and above. An open biopsy was taken from the edge of foot ulcer after informed consent of the patients. Age- and sex- matched control subjects without diabetes having wounds of extremities due to traumatic injury were included. Blood and tissue samples were collected. We included 10 diabetic and 6 non-diabetic patients of age 40 years and above foot ulcer subjects admitted from September 2011 to August 2012.

Ten type 2 diabetic patients (mean age 56, 9 men and 1 woman) and six non-diabetic patients (mean age 51, 6 men) were selected. Patients were eligible if they had: (1) a diabetic foot ulcer rated 1 to 3, stage A according to the University of Texas Wound Classification (2) a chronic wound (at least 30 days duration) ; (3) a wound area larger than 0.5cm^2 at inclusion.

Preparation of plasma and tissue sample

Collection of blood samples: Blood samples were collected from the patients by vein puncture and analyzed for glucose. The collected blood sample was centrifuged at 1800×g for 10 min at 37ºC to separate plasma, which was aspirated and stored at -20ºC until further analysis.

Harvesting of tissue biopsies: A 5 mm diameter biopsy were taken from the centre of the diabetic foot ulcer or the traumatic wound from non-diabetic subjects and immediately stored at -80ºC. The biopsies were collected from the first day of visit from the patients with diabetics and non-diabetic subjects in phosphate buffered solution and homogenized with Tris-Hcl buffer with 1500 rpm for 10 minutes followed by 8000 rpm for 20 minutes. Supernatant was stored in deep freezer for further procedure.

Gelatin Zymography

Gelatin zymography allows the detection and rapid identification of matrix metalloproteinase (MMP) activities in biological samples. Samples containing MMP, including plasma and tissue of diabetic and non-diabetic patients are subjected to separation by SDS-PAGE in a resolving gel containing a co-polymerized protease substrate such as gelatin. After being resolved in this gel, proteases are re-natured and act to hydrolyze the protein substrate in the gel matrix. The presence and relative activity of MMPs in the sample are thus determined by a decrease in Coomassie brilliant blue staining of the digested gelatin at the position of the MMPs. Diabetic foot ulcer patient's tissue samples were homogenized and centrifuged at 1500 rpm for 10 minutes and 8000 rpm for 20 minutes. A mixture of 4 proteins, from 16 to 68 kDa and human capillary whole blood were used for calibrating gelatin zymograms. Capillary blood was obtained by fingerstick puncture and prepared in nonreducing Laemmli SDS-PAGE sample buffer without heating [21].

Thiobarbituric acid reactive substances (TBARS)

The levels of lipid peroxidation in plasma and tissues were determined by the method of Niehaus and Samuelsson (1968) [38]. Malondialdehyde and other TBARS are quantitated by their reactivity with thiobarbituric acid (TBA) under acidic conditions. The reaction generates a pink coloured chromophore that can be measured in a colorimeter at 535 nm.

Reduced glutathione (GSH)

GSH in plasma and tissue were estimated by the method of Ellman (1959) [11]. This method was based on the development of yellow colour when DTNB [5, 5'dithiobis (2 – nitrobenzoic acid)] was added to compounds containing sulfhydryl groups.

α-Tocopherol (VITAMIN E)

Vitamin E content in tissue was estimated by the method of Baker *et al*. (1980) [4]. The method involves the reduction of ferric ions to ferrous ions by α-tocopherol and the formation of a red coloured complex upon reaction with 2, 2'-dipyridyl.

Ascorbic acid (VITAMIN C)

Ascorbic acid was quantified according to the method of Roe and Kuether (1943) [48]. Ascorbic acid is converted to dehydroascorbic acid in presence of thiourea, a mild reducing agent and then coupled with 2, 4- DNPH. The coupled DNPH is converted into a red coloured compound when treated with H_2SO_4, which was read at 520 nm.

Protein

The protein content of samples was estimated by the method of Lowry *et al.* (1951) [30]. Proteins react with Folin-Ciocalteau reagent to give a coloured complex. The colour formed is due to the reaction of alkaline copper with protein and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of the colour depends on the amount of these aromatic amino acids present.

Statistical analysis

Data were statistically analyzed using the Statistical Package for Social Sciences (SPSS) (version 16). Student's unpaired't' test was used to compare the mean levels of non-enzymatic antioxidants (plasma), TBARS (plasma and tissue) and glucose (plasma). Values were expressed as Mean ± S.E. "p" values less than 0.05 were considered statistically significant.

RESULTS:

The present study was carried out to compare the levels of MMPs and antioxidants in diabetic and non-diabetic foot ulcer patients. The major findings of our study are 1. diabetic foot ulcer patients have elevated glucose levels than non-diabetic foot ulcer patients. 2. levels of non-enzymatic antioxidants such as vitamin C, vitamin E and GSH were increased in diabetic foot ulcer patients than non-diabetic foot ulcer patients and 3. levels of MMP -2 and MMP-9 in chronic foot ulcer patients found to be increased compared to acute foot ulcer patients.

Plasma glucose

The levels of plasma glucose in diabetic foot ulcer and non-diabetic foot ulcer patients are presented in Table 4. Significant increase in glucose level was found in diabetic foot ulcer patients than non-diabetic foot ulcer patients.

Non-enzymatic antioxidants

Table 1 shows the levels of non-enzymatic antioxidants in plasma and tissue samples of diabetic and non-diabetic foot ulcer patients. Plasma level of GSH was decreased significantly in diabetic foot ulcer subjects compared to non-diabetic foot ulcer subjects. The level of GSH was found to be increased in the tissue samples of diabetic foot ulcer patients non-significantly compared to non-diabetic foot ulcer patients. Compared to non-diabetic subjects, levels of vitamin E and vitamin C in the plasma of diabetic foot ulcer patients were found to be increased than of non-diabetic foot ulcer patients.

Plasma and wound tissue TBARS levels

The levels of TBARS were found to be decreased in tissue and plasma samples of diabetic foot ulcer patients, compared to non-diabetic foot ulcer patients.

Expression of matrix metalloproteinases by substrate zymography

The levels of matrix metalloproteinases were measured using gelatin zymography. This technique identifies and separates the gelatinases, MMP-2 and MMP-9 in both latent and active forms.

Figure 1 shows the zymogram of plasma sample of diabetic and non-diabetic foot ulcer patients with respect to the pre-stained protein molecular weight marker. In the figure lane 1 represents the standard protein marker. Lane 2 represents diabetic foot ulcer patient's plasma sample. Lane 3 represents non-diabetic foot ulcer patient's plasma sample. Diabetic foot ulcer patient's plasma sample shows increased activity of MMP-9 than that of the non-diabetic foot ulcer patients.

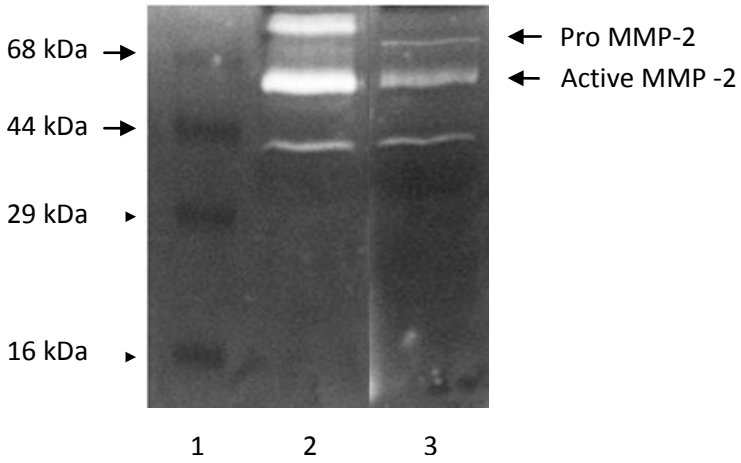
Figure 2 shows the relative band area of MMP-9 in diabetic and non-diabetic foot ulcer patient's plasma sample. Diabetic foot ulcer patient's plasma sample shows increased activity of MMP-9 than that of the non-diabetic foot ulcer patients. Zymogram shows distinct separation of the active pro forms of MMP-9 in plasma samples.

Figure 3 shows the zymogram of tissue sample of diabetic and non-diabetic foot ulcer patients with respect to the gelatinolytic activity of whole capillary blood. In the figure lane 2 and 3 represent tissue sample of diabetic foot ulcer patients. Lane 4 represents tissue sample of non-diabetic foot ulcer patient. The level of MMP-9 is found to be significantly increased in both diabetic and non-diabetic foot ulcer patients.

Figure 4 shows the relative arbitrary units of MMP-2 in diabetic and non-diabetic foot ulcer patient's plasma. Diabetic foot ulcer patient's plasma samples were found to have higher amount of MMP-2 than those of non-diabetic foot ulcer patients.

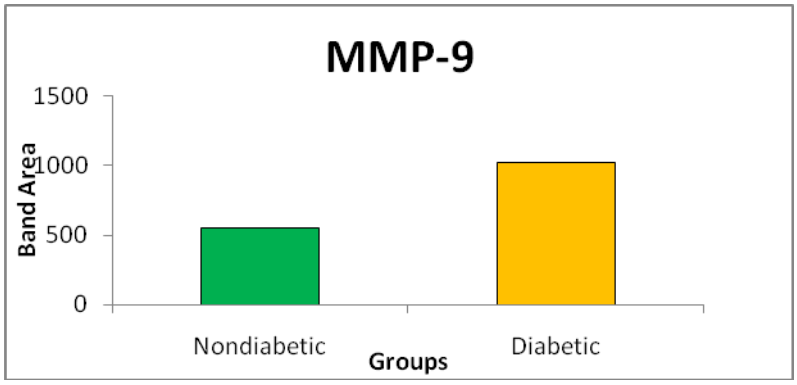
Figure 5 shows the zymogram of MMP-9 in diabetic and non-diabetic foot ulcer plasma samples with respect to the gelatinolytic activity of whole capillary blood. Diabetic foot ulcer patient's plasma sample shows increased activity of MMP-9 than that of the non-diabetic foot ulcer patients.

Figure 1: Representative gelatin zymogram of diabetic and non-diabetic foot ulcer patient's plasma samples.



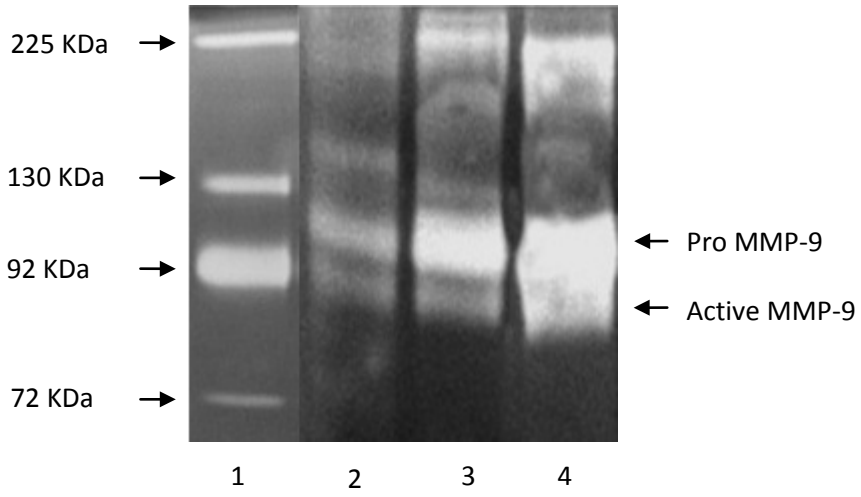
Lane 1: pre-stained protein molecular weight marker. Lane 2: plasma sample of diabetic foot ulcer patient. Lane 3: plasma sample of non-diabetic foot ulcer patient.

Figure 2: MMP- 9 in diabetic and non-diabetic foot ulcer plasma.



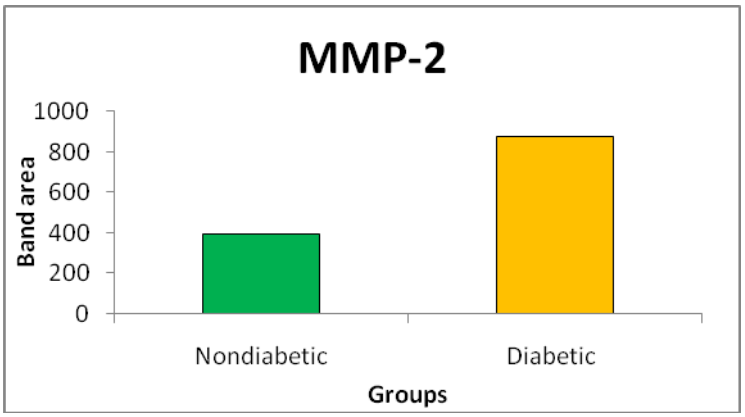
Average of total band area of the gelatinolytic activity of MMP 9 in the zymogram of diabetic and non-diabetic foot ulcer plasma sample is taken.

Figure 3: Representative gelatin zymogram of diabetic and non-diabetic foot ulcer patient's tissue samples.



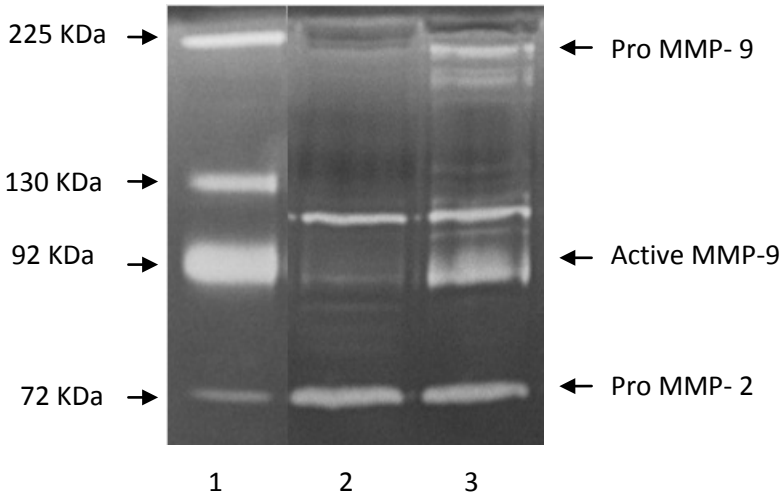
Lane 1: gelatinolytic activity of whole capillary blood. Lane 2 & 3: tissue sample of diabetic foot ulcer patients. Lane 4: tissue sample of non-diabetic foot ulcer patient.

Figure 4: MMP- 2 in diabetic and non-diabetic foot ulcer plasma.



Average of total band area of the gelatinolytic activity of MMP 9 in the zymogram of diabetic and non-diabetic foot ulcer plasma sample is taken.

Figure 5: Representative gelatin zymogram of diabetic and non-diabetic foot ulcer patient's plasma samples



Lane 1: gelatinolytic activity of whole capillary blood. Lane 2: plasma sample of non-diabetic foot ulcer patient. Lane 3: plasma sample of diabetic foot ulcer patient.

Table 1: Characteristics of diabetic and non-diabetic foot ulcer patients

Particulars	Diabetic foot ulcer	Non-diabetic foot ulcer
Number of patients	10	6
Sex	9 (M), 1 (F)	6(M)
Age (years)	56 ± 8	51 ± 11
Diabetes duration (years)	10 ± 5	-
Cigarette smoking (years)	2 ± 1	-
Drinking (years)	5 ± 4	-
Chewing betel nut (years)	3 ± 2	-

Table 2: Levels of glucose, non-enzymatic antioxidants and TBARS in diabetic and non diabetic plasma and tissue samples

Parameters	Non-Diabetic foot ulcer n=6	Diabetic foot ulcer n=10	Significance
Glucose (mg/dL)	109 ± 19.8	216 ± 126	P<0.05
GSH (t) (µg/g protein)	0.23 ± 0.21	0.43 ± 0.34	NS
GSH (p) (mg/ml)	1.31 ± 0.21	0.54 ± 0.34	P<0.05
TBARS(t) (nmoles/mg protein)	0.36 ± 0.28	0.13 ± 0.24	NS
TBARS (p)(nmoles/ml)	9.4 ± 3.4	4.4 ± 1.88	NS
Vitamin C (µg/ml)	0.31 ± 0.19	1.29 ± 0.96	P<0.05
Vitamin E (µg/ml)	2.79 ± 1.62	4.27 ± 3.66	P<0.05

Values are means ± SD. NS –Non-significant. t – tissue, p - plasma

DISCUSSION:

Non-healing ulceration is a serious complication of diabetes mellitus. Diabetic foot injuries often fail to heal because of persistently high concentrations of proinflammatory cytokines present in the wound, leading to high concentrations of proteases. These in turn degrade multiple growth factors, receptors, and matrix proteins that are essential for normal wound healing [2, 26, 32, 43, 51].

Increased glucose levels in the body end up in uncontrolled covalent bonding of aldose sugars to a protein or lipid without any normal glycosylation enzymes [1]. The stable advanced glycation end products (AGEs) then accumulate over the surface of cell membranes, structural proteins and circulating proteins. Formation of AGEs occurs on extracellular matrix proteins with slow turnover rate. AGEs alter the properties of matrix proteins such as collagen, vitronectin, and laminin through AGE-AGE intermolecular covalent bonds or cross-linking [1, 50, 9] AGE cross-linking on type I collagen and elastin results in increased stiffness.

Since patients with diabetic foot ulcer were provided vitamin supplements after surgery, they had an increased level of non-enzymatic antioxidants in circulatory fluids and thus the levels of TBARS in the tissue samples of diabetic foot ulcer patients found to be decreased when compared to non-diabetic foot ulcer patients.

Diabetic mice fed 0.5% vitamin C plus 0.5% vitamin E supplemented diet or 0.5% vitamin C, 0.5% vitamin E, plus 2.5% N acetyl cysteine supplemented diet showed 13 fold increase in vitamin C and E compared to those of the untreated diabetes mice [38]. Serum levels of antioxidant vitamins were significantly lower ($P < 0.05$) in diabetic subjects when compared with normal control individuals [41].

Vitamin C and E are non-enzymatic antioxidants which help in free radical scavenging and prevent development of diabetes [13].

Enzymatic antioxidant defences include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT). Non-enzymatic antioxidants are represented by ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione (GSH), carotenoids, flavonoids, and other antioxidants. Under normal conditions, there is a balance between both the activities and the intracellular levels of these antioxidants. This balance is essential for the survival of organisms and their health [31].

Estimation of lipid peroxidation is complicated by the large number of potential peroxidation products and by the reactivity of these metabolites. The most common technique for measuring lipid peroxide involves the use of thiobarbituric acid-reactive substances (TBARS) in serum. Increased levels of serum TBARS have been previously reported in patients with peripheral arterial disease, ishchemic heart disease, hypertension, and diabetes mellitus [17]. Serum levels of TBARS were significantly increased in all patients suffering from diabetes mellitus. The enhanced lipid peroxidation is

contributed to an increased formation of free radicals in diabetes mellitus. Earlier work by Kim *et al.,* 2007 and Odetti *et al.,* 2003 show decrease in TBARS levels in vitamin C or NAC (N-acetyl-L-cysteine) supplemented diabetic mice [22, 40].

Chronic ulcers are probably caused by an exaggeration of their inflammatory phase. This is supported by many studies where levels of MMPs were higher in the exudates of chronic wounds than in those of acute wounds, as in diabetic foot ulcers [26, 27]. The present study suggests that level of MMP- 2 and MMP -9 were significantly increased in diabetic and non-diabetic foot ulcer patients.

The concentrations of pro– and active–MMP-9 in the wound fluid obtained at presentation were significantly higher and those of TIMP-1 and TGF-1 significantly lower in ulcers that subsequently failed to heal than in ulcers that healed within 12 weeks. This pattern of increased MMP-9 in poorly healing ulcers was observed in varying types of diabetic foot ulcers, suggesting that it is more strongly linked with the healing process rather than with underlying etiology [56]. A balance between proteases and their inhibitors is necessary for a correct wound healing, and several studies [10, 55] have found elevated levels of proteases and reduced levels of inhibitors in chronic wounds. Increased levels of MMP-2 and MMP-9 could be demonstrated in various chronic wound liquids.
The pattern of MMP expression in normal wound healing is complex, with the concentration of the various MMPs changing according to the phase of healing. In the inflammatory phase, neutrophils and macrophages infiltrate the wound to phagocytose bacteria and there is an increase in expression of MMP-9. In the proliferative phase, fibroblasts predominate and the level of MMP-9 decreases, whilst the expression of other MMPs, particularly MMP-2 and MMP-1 begin to increase [54, 48, 35]. In some cells, high glucose concentration decreased MMP-2, MT1-MMP [34], but increased MMP-9, a pattern consistent with what is seen in diabetic wounds. Studies by others have shown in chronic wounds, including some diabetic wounds, elevated expression and activation of MMPs -2,-9 and -1 and decreased expression of TIMPs [28, 26, 52, 30, 45].

A balance between proteases and their inhibitors is necessary for a correct wound healing, and several studies have found elevated levels of proteases and reduced levels of inhibitors in chronic wounds [10, 55]. Increased levels of MMP-2 and MMP-9 could be demonstrated in various chronic wound liquids [53]. Ladewig *et al.,* 2002, demonstrated the ratio of MMP-9 to TIMP-1 as an important predictor for healing of chronic wounds, demonstrating an inverse correlation with the healing tendency of chronic pressure ulcers [24].

Higher concentrations of MMPs (MMP-2, -9, and -8) and reduced concentrations of inhibitors of MMPs (TIMP) in diabetic wounds compared with trauma lesions of a control group with normal glucose metabolism has been observed. In contrast to normal wound healing, an over expression of these proteases seems to support a delayed wound healing and lead to a failure of wounds to heal. Additionally, there is evidence of an imbalance between MMPs and TIMPs that significantly contributes to the pathogenesis of non-healing chronic lesions [26].

CONCLUSION:

The results of our study shown that plasma and tissue concentrations of MMP-9 and MMP-2 were increased in diabetic foot ulcer patients compared to non-diabetic foot ulcer patients. The mechanism of increased MMP-9 is uncertain. However, it is likely to be related to increased inflammation because MMP-9 is expressed mainly by neutrophils and macrophages, both cell types important to the inflammatory response [12].
The levels of non-enzymatic antioxidants were found to be high in the plasma and tissue samples of diabetic foot ulcer patients compared to non-diabetic foot ulcer patients since they were supplemented with antioxidant vitamins. Subjects with Type 2 diabetes in the study area have low levels of serum antioxidant vitamins. The deficiencies of these vitamins have been implicated in the development of diabetic late complications, such as cataract, nephropathy and neuropathy [42].

The elevated levels of antioxidants and decreased levels of TBARS in diabetes foot ulcer subjects in this study concludes that supplementation of antioxidant vitamins in therapeutic regimens could assist in management of diabetic and non-diabetic foot ulcer disease. Increased concentrations of MMP-9 and -2 diabetic foot ulcer subjects supports the previous studies that elevated levels of MMP-9 and -2 in chronic ulcer subjects delays wound healing process by degrading the extracellular matrix materials in an uncontrolled way. In conclusion we have performed the expression pattern on MMP-9 and -2 during healing process of diabetic and non-diabetic foot ulcers. This study provides evidence for the role of MMPs 2 and 9 in diabetic foot ulcer disease.

REFERENCES:

1. Alison Goldin, Joshua A. Beckman, Ann Mary Schmidt, Mark A. Creager. Circulation. 2006; 114: 597-605.
2. D.G. Armstrong, E.B. Jude. Journal of the American Podiatric Medical Association. 2002; In Press.

3. D.G. Armstrong, L.A. Lavery, A.J. Boulton. International Wound Journal. 2007; 4:79-86.
4. H. Baker, O. Frankel. Nutrition Reports International. 1980; 21: 531-536.
5. K. Bakker, W.H. Van Houtum, P.C. Riley. Current Diabetes Report. 2005; 5: 436-40.
6. T. Bjarnsholt , K. Kirketerp-Moller, P.O. Jensen, K.G. Madsen, R. Phipps, K. Krogfelt, N. Hoiby, M. Givskov. Wound Repair and Regeneration. 2008; 16:2-10.
7. E.J. Boyko, J.H. Ahroni, V .Stensel, R.C. Forsberg, D.R. Davignon, D.G. Smith. Diabetes Care. 1999; 22:1036-1042.
8. M. Brownlee. Annual Review of Medicine. 1995; 46: 223–234.
9. M. Brownlee. Nature. 2001; 414, 813-820.
10. E.C. Bullen, M.T. Longaker, D.L. Updike, R. Benton, D. Ladin, Z. Hou, E.W. Howard. Journal of Investigative Dermatology. 1995; 104:236–240.
11. M. Edmonds, A. Foster. The American Journal of Surgery. 2004; 187:25-28.
12. G.L. Ellman. Archives of Biochemistry and Biophysics. 1959; 82: 70 – 77.
13. V. Falanga. Lancet. 2005; 366: 1736–1743.
14. Fikret Karatas, Ihsan Halifeoglu, Mustafa Karatepe, Vahit Konar, Halit Canatan, Ramiz Colak. Arastirma. 2006; 20: 391 - 395
15. R.G. Frykberg, L.A. Lavery, H. Pham, C. Harvey, L. Harkless, A. Veves. Diabetes Care. 1998; 21:1714-1719.
16. R.G. Frykberg. American Family Physician. 2002; 66: 1655-1662.
17. R.G. Frykberg, T. Zgonis, D.G. Armstrong, V.R. Driver, J.M. Giurini, S.R Kravitz. Journal of Foot and Ankle Surgery. 2006; 45:1–66.
18. Y. Fujiwara. Journal of Occupational Medicine and Toxicology. 2002; 18: 3-10.
19. R. Gadepalli, B. Dhawan, V. Sreenivas, A. Kapil, A.C. Ammini, R. Chaudhry. Diabetes Care. 2006; 29:1727-32.
20. W.H. Goodson, T.K. Hunt. The Journal of surgery, gynecology and obstetrics. 1979; 149: 600– 8.
21. Gregory S. Makowski, Melinda L. Ramsby. Analytical Biochemistry. 1996; 236: 353-356.
22. W.J. Jeffcoate, K.G. Harding. Lancet. 2003; 361:1545-51.
23. A. Kashiwagi. Nippon Rinsho. 2010; 68: 777-87.
24. S.K. Kim, J.E. Do, H.Y. Kang, E.S. Lee, Y.C. Kim. European Journal of Dermatology. 2007; 17:537-8.
25. S. Kumar, H.A. Ashe, L.N. Parnell, D.J. Fernando, C. Tsigos, R.J. Young, J.D. Ward, A.J. Boulton. Diabetic Medicine. 1994; 11:480-484.
26. G.P. Ladwig, M.C. Robson, R. Liu, M.A. Kuhn, D.F. Muir, G.S. Schultz. Wound Repair Regeneration. 2002; 10:26–37.
27. G.S. Lazarus. Archives of Dermatological Research. 1994; 130: 1539-42.
28. R. Lobmann, A. Ambrosch, G. Schultz, K. Waldmann, S .Schiweck, H. Lehnert. Diabetologia. 2002; 45:1011-1016.
29. R. Lobmann, A. Ambrosch, G. Schultz, K. Waldmann, S. Schiweck, H. Lehnert. Diabetic care. 2005; 28: 461-471.
30. R. Lobmann, C. Zemlin, M. Motzkau, K. Reschke, H. Lehnert. Journal of Diabetes and its Complications. 2006; 20: 329-335.
31. O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randell. Journal of Biological Chemistry. 1951; 193: 265-275.
32. M. Muller, C. Trocme, B. Lardy, F. Morel, S. Halimi and P. Y. Benhamou. Diabetic Medicine. 2008; 25: 419–426.
33. Marian Valko, Dieter Leibfritz, Jan Moncola, Mark T.D. Cronin, Milan Mazura, Joshua Telser. The International Journal of Biochemistry & Cell Biology. 2007; 39: 44–84
34. B.A. Mast, G.S. Schultz. Wound Repair and Regeneration. 1996; 4:411-20.
35. A.A. Mavian, S. Miller, R.R. Henry. Postgraduate Medicine. 2010; 122:106-17.
36. S.V. McLennan, S.Y. Martell, D.K. Yue. Diabetologia. 2000; 3: 642-648.
37. R. Mohan, S.K. Chintala, J.C. Jung. Journal of Biological Chemistry. 2002; 277:2065-2072.
38. S.E. Moss, R. Klein, B.E. Klein. Diabetes Care. 1999; 22:951-959.
39. S.E. Moss, R. Klein, B.E. Klein. Archives of Internal Medicine. 1992; 152:610-616.
40. Na-Young Park, Yunsook Lim. Nutrition & Metabolism. 2011; 8:1-9.
41. A. Neu, S. Ehehalt, A. Willasch, M. Kehrer, R. Hub, M.B. Ranke. Pediatric Diabetes. 2001; 2:147-53.
42. W.G. Niehaus Jr, B. Samuelsson. European Journal of Biochemistry. 1968; 17: 126-130.
43. P. Odetti, C. Pesce, N. Traverso, S. Menin, E.P. Maineri, L. Cosso, S. Valentini, S. Patriarca, D. Cottalasso, U.M. Marinari, M.A. Pronzato. Diabetes. 2003; 52:499-505.
44. I. Onyesom, J.E. Agho, H.E. Osioh. African Journal of Pharmacy and Pharmacology. 2011; 5: 1787-1791.
45. L. Packer. Journal of Free Radicals in Biology and Medicine. 2002; 20: 1020-1032.
46. J.B. Petri , S. Konig , B. Haupt , U.F. Haustein , K. Herrmann . Experimental Dermatology. 1997; 6:133-9.

CODEN (USA): JBPRAU

47. S.D. Ramsey, K. Newton, D. Blough, D.K. McCulloch, N. Sandhu, G.E. Reiber, E.H. Wagner. Diabetes Care. 1999; 22:382-387.

48. Rayment, A. Erin, Upton, Zee, Shooter, K. Gary. British Journal of Dermatology. 2008; 158: 951-961.

49. G.E. Reiber, E.J. Boyko, D.G. Smith. Diabetes in America. 1999; 2nd ed, pp 409-427.

50. J.H. Roe, C.A. Kuether. Journal of Biological Chemistry. 1943; 147: 399-407.

51. T. Salo, M. Makela, M. Kylmaniemi, H. Autio-Harmainen, H. Larjava. Laboratory Investigation. 1994; 70:176-182.

52. N. Singh, D.G. Armstrong, B.A. Lipsky. The Journal of the American Medical Association. 2005; 293:217-28.

53. R.K. Singh, M. Gutman , R. Reich , M. Bar-Eli . Cancer Research. 1995; 55:3669-74.

54. R.W. Tarnuzzer, G.S. Schultz. Wound Repair and Regeneration. 1996; 3: 321–325.

55. N.J. Trengove, M.C. Stacey, S. MacAuley. Wound Repair Regeneration. 1999; 6: 442-452.

56. M. Vaalamo, M. Weckroth, P. Puolakkainen, J. Kere, P. Saarinen, J. Lauharanta, U.K. Saarialho- Kere. British Journal of Dermatology. 1996; 135: 52–59.

57. S.J. Wall, D. Bevan, D.W. Thomas, K.G. Harding, D.R. Edwards, G. Murphy. Journal of Investigative Dermatology. 2002; 119:91-98.

58. A.B. Wysocki, L. Staiano-Coico, F. Grinnell. Journal of Investigative Dermatology. 1993; 101:64–68.

59. L. Xu, S.V. McLennan, L. Lo, A. Natfaji, T. Bolton, Y. Liu, S.M. Twigg, D.K. Yue. Diabetes Care. 2007; 30:378–380.

60. Yu Liu, Danqing Min, Thyra Bolton, Vanessa Nube, Stephen M. Twigg, Dennis K. Yue. Diabetes care. 2009; 32: 117-119.