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ROLE OF TNF-A AND INTERFERON-GAMMA IN DIABETES AND NON-DIABETIC FOOT ULCER PATIENTS

Medicine

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ABSTRACT

Diabetic foot ulcer is a major complication of diabetes mellitus, and probably the major component of the diabetic foot. In diabetic wound healing impaired fibroblast proliferation has been linked to increased levels of TNF-α .

The aim of the study to find any relationship of Cytokines- TNFα and γ INTERFERON in DM with Foot Ulcer.

We found that over expression of Interferon gamma level was significantly higher in Type2 DM with Foot Ulcer than Healthy Control (t=12.8692). T-test showed that mean Interferon gamma of Type2 DM with Foot Ulcer patients had significantly higher than others. Mean of TNF α level was significantly higher in Type2 DM with Foot Ulcer than Healthy Control (t=10.6536). T-test showed that mean TNF α of Type2 DM with Foot Ulcer patients had significantly higher than others.

High level of Cytokines like TNF-α and Interferon-gamma are also responsible for the development of late Diabetic complications.

KEYWORDS

Cytokines, TNF-α, Interferon-gamma, Diabetes, non-diabetic foot ulcer.

INTRODUCTION

Diabetic foot ulcer is a major complication of diabetes mellitus, and probably the major component of the diabetic foot. Wound healing is an innate mechanism of action that works reliably most of the time. A key feature of wound healing is stepwise repair of lost extracellular matrix (ECM) that forms the largest component of the dermal skin layer.[1]But in some cases, certain disorders or physiological insult disturbs the wound healing process. Diabetes mellitus is one such metabolic disorder that impedes the normal steps of the wound healing process. Many studies show a prolonged inflammatory phase in diabetic wounds, which causes a delay in the formation of mature granulation tissue and a parallel reduction in wound tensile strength.[2]Treatment of diabetic foot ulcers should include: blood sugar control, removal of dead tissue from the wound, wound dressings, and removing pressure from the wound through techniques such as total contact casting. Surgery in some cases may improve outcomes.[3] Hyperbaric oxygen therapy may also help but is expensive.[3]It occurs in 15% of people with diabetes,[4] and precedes 84% of all diabetes-related lower-leg amputations.[5]

The inflammatory stage of wound repair occurs shortly after tissue damage. After acute injury, platelets and neutrophils are released passively from disrupted blood vessels. The formation of a fibrin clot provides a temporary scaffold for infiltration of inflammatory cells. A large number of growth factors are important in stimulating and coordinating cellular events that occur during normal wound healing. [6] Among them, cytokines and chemokines are especially noted because of their roles in promoting inflammation, angiogenesis, leukocyte recruitment, recruitment of stem cells, and epithelialization. Proinflammatory cytokines that are elevated shortly after wounding both in human wounds, and animal wound models include IL-1α, IL-1β, IL-6, IL-12, and TNF-α.[7,8.]

In normal wound healing the highest levels of TNF-α are seen from 12 to 24 h after wounding. During the early phase of wound repair, it is predominantly expressed in polymorphonuclear leukocytes, and later by macrophages. It is also expressed in the hyperproliferative epithelium at the wound edge. TNF-α contributes to the stimulation of fibroblasts and keratinocytes the expression of growth factors and up-regulation of antimicrobial defenses. TNF-α levels are elevated in diabetes in part through increased oxidative stress that promotes inflammation. Other factors may contribute to this elevation including the down regulation of CD33 that inhibits cytokine production. TNF-α is found threefold higher in diabetic mouse wounds than wounds in normal mice and threefold higher found in wound fluid from nonhealing venous leg ulcers than in healing ulcers. Chronic gastric ulcers are also associated with increased TNF-α.[9]

In diabetic wound healing impaired fibroblast proliferation has been linked to increased levels of TNF-α . Inhibiting TNF in vivo significantly increases the number of proliferating fibroblasts but it has a little effect on fibroblast proliferation in normoglycemic mice. Apoptosis of fibroblasts in diabetic mice is significantly higher than in normoglycemic counterparts, and apoptosis is high in skin biopsies from diabetic foot ulcers. TNF stimulates apoptosis of fibroblasts, keratinocytes, and endothelial cells in vitro. A cause-and-effect relationship has been established between the treatment of TNF blocker and reduced apoptosis which was elevated in diabetic healing. Diabetes also impairs the migration of fibroblasts and keratinocytes. High levels of TNF-α inhibit cell migration. This may occur by increasing the level of Smad 7 and inhibiting the activation of the Smad 2/3 [10]The neutralization of TNF in the diabetic wounds improves wound angiogenesis and closure. Blocking TNF reduces the overproduction of small noncoding RNAs such as miR-200b in the diabetic wounds, which improves the expression of globin transcription factor-binding protein 2 (GATA2) and vascular endothelial growth factor receptor 2 (VEGFR2), both of which promote angiogenesis.[11]

The ability of cells at the wound site to respond to insulin is reduced in diabetic wounds. Insulin insensitivity occurs when the response to insulin is reduced. Long-term treatment of cells with TNF-α contributes to reduced insulin sensitivity. Insulin receptor expression in proliferating keratinocytes at the wound margins and in granulation tissue is reduced in diabetic mice but enhanced with anti-TNF-α antibody treatment. The effect of neutralization of TNF-α on insulin sensitivity may be involved in inhibiting the effects of TNF-α on the downregulation of GLUT4 genes that are required for normal insulin action, the downregulation of PPARγ which is an important insulin-sensitizing nuclear receptor, and the upregulation of Ser phosphorylation of IRS-1 that results in a net decrease in insulin receptor-mediated signaling. Thus, an important component of impaired diabetic wound healing may be due to the reduced sensitivity of cells that participate in the wound healing process to insulin stimulation, which is mediated in part by high levels of TNF.[12]

The aim of the study to find any relationship of Cytokines- TNFα and γ INTERFERONin DFU.

MATERIALS AND METHODS

INCLUSION CRITERIA

1. Vascular foot ulcers
2. Neuropathic foot ulcers
3. Infective foot ulcers
4. Healthy Control.

EXCLUSION CRITERIA

1. Traumatic Ulcers
2. Steroid Induced Ulcers
3. Malignant Ulcers
4. Radiation Ulcers
5. Skin diseases

SAMPLE DESIGN

1. Healthy Control, 50
2. Diabetic population with foot ulcer, 50
3. Diabetic population without foot ulcer, 50
4. Non-diabetic population with foot ulcer, 50

Study group:

1. Healthy Control, 50 persons
2. Diabetic population with foot ulcer, 50patients
3. Diabetic population without foot ulcer, 50patients
4. Non-diabetic population with foot ulcer, 50patients

STATISTICALANALYSIS:

For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS (version 24.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5. Data had been summarized as mean and standard deviation for numerical variables and count and percentages for categorical variables. One-way analysis of variance (one-way ANOVA) was a technique used to compare means of three or more samples for numerical data (using the F distribution). A chi-squared test ($\chi 2$ test) was any statistical hypothesis test wherein the sampling distribution of the test statistic is a chi-squared distribution when the null hypothesis is true. Without other qualification, 'chi-squared test' often is used as short for Pearson's chi-squared test. Unpaired proportions were compared by Chi-square test or Fischer's exact test, as appropriate.

p-value ≤ 0.05 was considered for statistically significant.

RESULTS

In type2 DM with foot ulcer, the mean age (mean±s.d.) of patients was 54.6200± 10.8438 years. In non-diabetic foot ulcer, the mean age (mean±s.d.) of patients was 42.3600± 13.4661 years. In type2 DM without foot ulcer, the mean age (mean±s.d.) of patients was 50.5000± 11.3986 years. In Healthy Control, the mean age (mean±s.d.) of patients was 45.1800± 15.2472 years. Distribution of mean age vs. group was statistically significant (p<0.0001). Association of sex vs. group was statistically significant (p<0.0001).

We found that in type2 DM with foot ulcer, higher number of patients 16(32.0%) had house wife. In non diabetic foot ulcer, higher number of patients 28(56.0%) had house wife. In type2 DM without foot ulcer, higher number of patients 26(52.0%) had house wife. In Healthy Control, higher number of patients 29(58.0%) had house wife. Association of occupation vs. group was not statistically significant (p=0.0002).

We found that in type2 DM with foot ulcer, higher number of patients 26(52.0%) had secondary. In non-diabetic foot ulcer, higher number of patients 28(56.0%) had secondary. In type2 DM without foot ulcer, higher number of patients 26(52.0%) had secondary. In Healthy Control, higher number of patients 22(44.0%) had secondary. Association of educational qualification vs. group was statistically significant (p=0.0105).

It was found that in type2 DM with foot ulcer, higher number of patients 46(92.0%) had married. In non diabetic foot ulcer, higher number of patients 48(96.0%) had married. In type2 DM without foot ulcer, higher number of patients 49(98.0%) had married. In Healthy Control, higher number of patients 48(96.0%) had married. Association of married vs. group was not statistically significant (p=0.4499).

We found that in type2 DM with foot ulcer, 26(52.0%) patients had family history. In non-diabetic foot ulcer, 13(26.0%) patients had family history. In type2 DM without foot ulcer, 27(54.0%) patients had family history. In Healthy Control, 12(24.0%) patients had family history. Association of family history &hereditary vs. group was statistically significant (p=0.0009).

It was found that in type2 DM with foot ulcer, higher number of patients 32(64.0%) had no addiction. In non diabetic foot ulcer, higher number of patients 38(76.0%) had no addiction. In type2 DM without foot ulcer, higher number of patients 43(86.0%) had no addiction. In Healthy Control, higher number of patients 50(100.0%) had no addiction. Association of addiction vs. group was statistically significant (p<0.0001).

We found that in type2 DM with foot ulcer, the mean Interferon gamma (mean±s.d.) of patients was 48.0118 ± 15.7053. In non-diabetic foot ulcer, the mean Interferon gamma (mean±s.d.) of patients was 37.0455 ± 14.9428. In type2 DM without foot ulcer, the mean Interferon gamma (mean±s.d.) of patients was 45.6680 ± 17.0887. In Healthy Control, the mean Interferon gamma (mean±s.d.) of patients was 13.7678 ± 10.3621. Distribution of mean Interferon gamma vs. group was statistically significant (p<0.0001).

It was found that in type2 DM with foot ulcer, the mean TNF α (mean±s.d.) of patients was 50.9426 ± 24.5381.In non diabetic foot ulcer, the mean TNF α (mean±s.d.) of patients was 45.3804 ± 22.2435.In type2 DM without foot ulcer, the mean TNF α (mean±s.d.) of patients was 40.7898 ± 14.2955.In Healthy Control, the mean TNF α (mean±s.d.) of patients was 11.8054 ± 8.5235.Distribution of mean TNF α vs. group was statistically significant (p<0.0001).

DISCUSSION

Diabetic foot ulcer is the common dreadful complication of diabetes mellitus. The lifetime prevalence of foot ulceration is about 15%. [13] Macro and microvascular involvement and neuropathy plays a major role in the pathophysiology of diabetic foot ulcers. [14] According to the Diabetes Atlas 2013 published by the International Diabetes Federation, the number of people with diabetes in India currently is 65.1 million, which is expected to rise to 142.7 million by 2035.[15] Mean age of the study population was 51 years, which is in par with the previous studies in India. [16]

We found that mean age was higher in type2 DM with foot ulcer patients than others and that was statistically significant (p<0.0001).Present study found that male had more prevalence in Type2 DM with Foot Ulcer and it was statistically significant (p<0.0001). In type2 DM with foot ulcer, higher number of patients 16(32.0%) were house wives. In non-diabetic foot ulcer, higher number of patients 28(56.0%) were house wives. In type2 DM without foot ulcer, higher number of patients 26(52.0%) were house wives. In healthy control, higher number of patients 29(58.0%) were house wives. Association of occupation vs. group was not statistically significant (p=0.0002).

In type2 DM with foot ulcer, higher number of patients 26(52.0%) had secondary education. In non-diabetic foot ulcer, higher number of patients 28(56.0%) had secondary education. In type2 DM without foot ulcer, higher number of patients 26(52.0%) had secondary education. In Healthy Control, higher number of patients 22(44.0%) had secondary. Association of educational qualification vs. group was statistically significant (p=0.0105). Association of married vs. group was not statistically significant (p=0.4499). In type2 DM with foot ulcer, 26(52.0%) patients had family history. In non-diabetic foot ulcer, 13(26.0%) patients had family history. In type2 DM without foot ulcer, 27(54.0%) patients had family history. In Healthy Control, 12(24.0%) patients had family history. Association of family history vs. group was statistically significant (p=0.0009). Higher proportion patients had no addiction in all groups that is statistically significant.

In diabetic wound healing impaired fibroblast proliferation has been linked to increased levels of TNF-α .[17] Wound healing requires the transition of basal and suprabasal keratinocytes from a sedentary phenotype to a migratory and hyper proliferative phenotype. The epithelialization process involves local keratinocytes at the wound edges and epithelial stem cells from hair follicles or sweat glands. [18,19] Keratinocytes are a major source of growth factors such as TGF-β, VEGF, EGF, KGF, and TGF-α that stimulate fibrogenesis and

angiogenesis in adjacent tissue .[20,21] The activation of one of the AGE receptors, (receptor for AGEs), RAGE causes the upregulation of the transcription factor nuclear factor-kappa B (NF-kappa B) and its target genes such as intercellular adhesion molecule-1 (ICAM-1), VEGF, IL-1α, IL-6, and TNF-α. Mice fed with high levels of AGE display impaired wound closure. [22]

General expression of TNF-α receptors by a wide variety of cells and tissues suggests that TNF-α is involved in a number of biological activities. Besides its pro-inflammatory role, TNF-α has other functions such as promotion of T cell proliferation in vitro [23], prevention of T cell deletion induced by superantigens , and it critically influences germinal centre formation following immunization [24]. In pathological circumstances, all of these properties, which contribute to the establishment, maintenance, or accentuation of specific immune responses, could aberrantly end in tissue injury. Interferon-gamma (IFN-γ), also known as type II interferon or macrophage-activating factor (MAF), was originally identified due to its antiviral activity. [25] It had been reported that VEGF gene polymorphisms were associated with T2DM, as well as its complications.[26]

We found that over expression of Interferon gamma level was significantly higher in Type2 DM with Foot Ulcer than Healthy Control (t=12.8692). T-test showed that mean Interferon gamma of Type2 DM with Foot Ulcer patients was significantly higher than others. Mean of TNF α level was significantly higher in Type2 DM with Foot Ulcer than Healthy Control (t=10.6536). T-test showed that mean TNF α of Type2 DM with Foot Ulcer patients were significantly higher than others.

CONCLUSION

High level of TNF-α and Interferon-gamma inhibit angiogenesis and cell proliferation and migration in diabetic wounds and increases apoptosis levels. TNF inhibition attenuates the impact of diabetes-enhanced TNF-α, which offers potentially new therapeutic avenue for treatment of abnormally diabetic wounds healing. Diabetes-enhanced and prolonged expression of TNF-α and Interferon-gamma also contributes to impair healing. In our study, we discuss the abnormal cell responses in diabetic wound healing and the contribution of TNF-α and Interferon-gamma. Cytokines like TNF-α and Interferon-gamma are also responsible for the development of late Diabetic complications.

Table 1: Distribution of mean Interferon gamma

	Group	Number	Mean	SD	Minimum	Maximum	Median	p-value
Interferon gamma	Type2 DM with foot ulcer	50	48.0118	15.7053	22.1200	89.4500	46.3100	<0.0001
	Non diabetic foot ulcer	49	37.0455	14.9428	15.3400	89.4500	35.6400	
	Type2 DM without foot ulcer	50	45.6680	17.0887	23.1800	71.2600	40.9700	
	Healthy Control	50	13.7678	10.3621	2.3400	35.2600	13.3400	

	T Statistic	P-value
Type2 DM with Foot Ulcer vs Healthy Control	12.8692	<0.0001
Type2 DM without Foot Ulcer vs Healthy Control	11.2869	<0.0001
Non diabetic foot ulcer vs Healthy Control	9.0223	<0.0001

Table 2: Distribution of mean TNF α

	Group	Number	Mean	SD	Minimum	Maximum	Median	p-value
TNF α	Type2 DM with foot ulcer	50	50.9426	24.5381	25.6500	102.0800	40.8700	<0.0001
	Non diabetic foot ulcer	50	45.3804	22.2435	23.1500	102.0800	37.9800	
	Type2 DM without foot ulcer	50	40.7898	14.2955	21.3600	63.2100	34.2600	
	Healthy Control	50	11.8054	8.5235	3.6500	38.7500	9.6500	

	T Statistic	P-value
Type2 DM with Foot Ulcer vs Healthy Control	10.6536	<0.0001
Type2 DM without Foot Ulcer vs Healthy Control	12.3140	<0.0001
Non diabetic foot ulcer vs Healthy Control	9.9666	<0.0001

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