Assessment of the S100 Protein of the Common Peroneal Nerves in Diabetic Patients

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Abstract

The present work designed to study the Immunohistochemical changes of the common peroneal nerves of 30 diabetic patients (8 females and 22 males), their age ranging between (55-75) years and identical samples from 30 un diabetic cadavers (4 females and 26 males) newly imported to department of forensic medicine, at age of (25-50) years as control group. The multiple regression analysis showed significant effect, of age, male gender and duration of diabetes on the development of neuropathy. The protein S100 was significantly depleted in common peroneal nerves samples taken from diabetic patients. The male gender, age progression and to a lesser extent the duration of the disease observed to be risk factors aggravating the depletion of the S100 protein. The present study concluded that neurological factors were simultaneously responsible for the pathogenesis of diabetic peripheral neuropathy, and the age, male gender and a lesser extent the diabetic period may act as risk factors.

Introduction

Anatomy of the common peroneal nerve: arises from the fourth and fifth lumbar and the first and second sacral nerves. It is one of the constituents of the sciatic trunk. At the bifurcation of the sciatic trunk the common peroneal nerve gradually passes laterally away from the tibial nerve and passing superficial to the origin of the lateral head of gastrocnemius, immediately dorsal to the tendon of the biceps muscle, enters the leg [1].

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Eckert et al (2004) commented upon S-100 proteins and said that; they are of interest as mediators of calcium-associated signal transduction and undergo changes in sub cellular distribution in response to extracellular stimuli and recently, S-100 proteins have received increasing attention due to their close association with several human diseases including cardiomyopathy, neurodegenerative disorders and cancer. S100 proteins are expressed in peripheral neurons. Most of them express S-100 α protein, and a subpopulation of sensory neurons in dorsal root ganglia contains S-100 β protein or S-100 α plus S-100 β proteins[2].

Marenholz et al (2004) found that the expression of S-100 by peripheral glial cells seems to be a distinctive fact of these cells, independently of their localization and their ability [3].

Male patients with type 2 diabetes may develop diabetic polyneuropathy earlier than female patients. Diabetic neuropathy can occur at any age but is more common with increasing age and severity and duration of diabetes [4].

The relationship among Schwann cells, axons, and the perineurial barrier emphasize the key role in normal functions of the nerve [5]. Reduced nerve perfusion is an important factor in the etiology of diabetic neuropathy [6].

Several studies have demonstrated that subjects with impaired glucose tolerance (IGT) develop peripheral neuropathy. Indeed, subjects with IGT or type II diabetes and with peripheral neuropathy showed a comparable decrease in myelinated nerve fiber density. Both metabolic and vascular factors are important in the pathogenesis of peripheral neuropathy in diabetes. The exact relationship between the severity of hyperglycemia and these factors remains to be established. Diabetic patients without evidence of neuropathy demonstrate endoneurial microangiopathy [7].

Nerve biopsies in diabetic neuropathy demonstrate multifocal fiber loss. Diabetic nerves have been shown to exhibit increased pathological vulnerability to ischemia [8].

Aims of the Study

This study is carried out to

1- Determine the Immunohistochemical localization of S100 protein and its role in the pathogenesis of diabetic peripheral neuropathy.

2. To evaluate multiple risk factors for diabetic peripheral neuropathy.

Materials and Methods

One cm of the common peroneal nerve (CPN) taken from proximal healthy part of the amputated legs of 30 diabetic patients (22 male and 8 female) at the (55–75 years) in the surgical theater of different hospitals in Erbil city. Homologue nerve samples taken from 30 (un diabetic) cadavers (4 female and 26 male) that had no history of diabetes and newly imported to the forensic medicine (Rizgary Teaching Hospital) as control group at (25–50 years). Patients and cadavers with previous history of neurovascular disease were excluded from the study. The skin incised down the middle of the popliteal fossa to posterior leg. By using blunt dissection the common peroneal nerve is followed laterally along the superolateral border of the popliteal fossa. The common peroneal nerve parallels the biceps femoris tendon and passes superficial to the lateral head of gatrocnemius muscle [9], one centimeter length was taken from the original nerve samples , fixed in formal saline solution [10] for about 24 hours, processed automatically using (Sakura Automatic Tissue Processor made in US). After 24 hour the samples embedded in molten paraffin wax. Blocks then made available for microtomy using (Leitz rotatory microtome). Sections of the tissues were cut at five micrometer to be stained with:

1- Haematoxyline and eosin (H & E) [10].

2- Immunohistochemistery using anti S 100 antibody from Dako Company, EnVision®+Dual link system-HRP(DAB+) (Polyclonal Rabbit Anti S100, ready to use).

Positive expression of immunostaining gave clear cut nuclear staining of brown color. Positive cells were determined by counting 1000 Schwann cells. All significantly stained cells were considered positive and divided by 10 to acquire the percentage (immunostaining index); at least 10 HPFs were measured for each case for the purpose of scoring.

The extent of S100 immunostaining was assessed; Negative (cut off point) when S100 index < 10%, weak positive (cut off point) when S100 index ≤ 11-49% and strong positive (cut off point) when S100 index ≥ 50[11].

Statistical Analysis

Statistical analysis was made using statistical package for social sciences (SPSS) computer software version 15. The following tests were used: ANOVA test, independent samples t test, simple linear correlation and multiple regression analysis. A p value of ≤ 0.05 was considered as statistically significant.

Results

A special Immunohistochemical technique was applied for the location of S100 protein in both CPN from diabetic and non diabetic samples. The result showed that the amount of the protein was markedly depleted from CPN of the diabetics when compared to control group (Figures 1, 2, table 1)

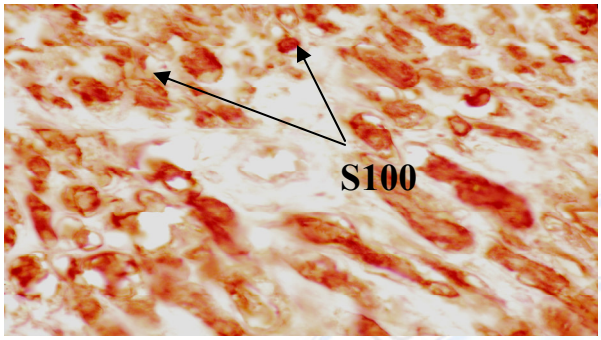

Figure (1): Section from CPN of a normal control specimen with IHC stain for S100 protein (arrows) showing normal activity. X1000.

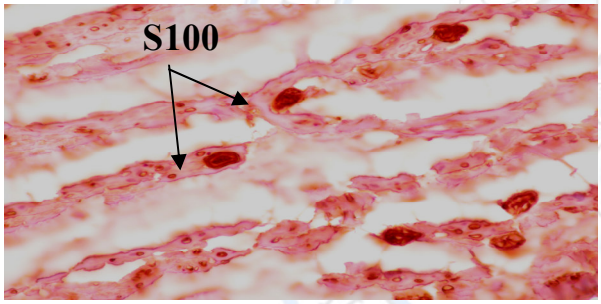

Figure (2): Section from CPN of a diabetic specimen with IHC stain for S100 Protein showing marked reduction in the activity of S100 (arrows).. X1000.

Effect of gender on S100 protein expression CPN

S100 protein expression in the CPN affected by gender in diabetics; it was severely depleted in male compared to female diabetics. While in control group there was no significant change in both nerves concerning the localization of this protein (Table 2).

Table (1): S100 protein expression in diabetes and control groups

Group		N	Mean ±SD	95% confidence interval of the difference	P
CPN	Diabetic	30	15.17 ±10.13	-2.37 to -63.63	< 0.001
	Control	30	83.83 ±6.52		

Table(2): S100 protein expression by gender

Group		Gender	N	Mean ±SD	95% confidence interval of the difference	p
Diabetic	CPN	Male Female	22 8	12.05±8.12 26.25±6.94	-20.83 to -7.58	< 0.001
Control	CPN	Male Female	26 4	83.85±6.05 83.75±10.31	-7.21 to 7.40	0.98

The correlation between the S100 expression with age and duration of diabetes:

The result of the present study showed that there was a significant negative correlation between the localization of S100 protein and age in among the diabetics i.e. the amount of S100 protein reduced with increasing age, although it was not correlated with age in control group. However it was reduced to a lesser amount in response to the increased duration of the disease than with age (Table 3).

Table (3): correlation between S100 protein percentage in diabetic group with age and duration of diabetes in years:

		S 100 protein	N	R	sig
age	Diabetic group	CPN	30	-0.63**	< 0.001
	Control group	CPN	30	-0.21	0.28
Duration of diabetes		CPN	30	-0.45*	0.01

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

The effect of age, gender and the group on the S100 protein expression in the CPN:

The interaction of age, gender and group was studied on the localization of S100 protein in the CPN. A statistically significant relationship was established with all these factors. These factors were independent predictors for the localization of this protein (Tables 4).

Table (4): The effect of age gender, and group on CPN S100 protein expression as dependent variable

	Unstandarized Coefficients		Standarized Coefficients	t	p
Model 1	B	SD	Beta		
(Constant)	-8.45	13.78		-0.61	0.54
age	-0.64	0.15	-0.26	-4.28	<0.001
gender	9.67	2.22	0.11	4.35	<0.001
group	52.76	4.23	0.75	12.48	<0.001

Dependent variable CPN S100 protein

Adjusted R square 0.96

Discussion

There is limited literature regarding the localization of S100 protein in human samples while studies on animal tissues, are also rare. The results of the present study is in agreement with (Shearman and Franks, 1987), (Mata et al 1990), and (Bhatheja and Field 2006) in that showed that diabetics affected by significant depletion of the S100 protein within the common peroneal nerve [12,13,14].

By reviewing the function of S100 protein on Schwann cell, the effect of diabetes on the localization of S100 protein can be explained. Programmed cell death in Schwann cells of the peripheral nervous system is activated by diabetes and hyperglycemia, which leads to Schwann cell dysfunction and reduced myelin production by alterations in the mitochondrial function. The immunoreactivity of S100 protein in Schwann cells is dependable on the thickness of myelin sheath; consequently it will cause a decrease in S100 protein localization in cases of diabetic peripheral neuropathy. Lastly with advancing age there will be a decrease in the production of the myelin forming proteins via Schwann cell in the peripheral nervous system. In the diabetic peripheral neuropathy there is a progressive loss of myelin sheath,

consequently leading to the decreased localization of the S100 protein [15,16,19]. No other research studies have specifically examined the S 100 protein in the peripheral nerve and their relation to age and sex or other factors like alcohol and smoking.

The present study showed that S100 protein expression in the CPN was affected by gender, age and to a lesser extent by duration of diabetes. In diabetics; it was severely depleted in males compared with females. There was a moderate negative correlation with age and duration of the disease. One explanation for that could be the time difference in the activity of this protein between male and female as has been documented by Nogueira et al (2009) who showed rhythmic daily expression, the female and male rats showed opposite cycles. Females presented the highest value at the beginning of the rest phase (5:00 h), while in males the maximum value appeared in the beginning of the motor activity period (21:00 h). While in control group there was no significant change in both nerves concerning the localization of this protein either for gender or age group. This could be explained by the fact that with increasing age and presence of diabetes and more duration of hyperglycemia there will be destruction of the myelin sheath and activated programmed cell death and decreased myelin forming proteins[17].

Diabetic state is the most important factor that affects mainly on the localization of S100 protein by the pathological effects of the prolonged exposure of the Schwann cells to hyperglycemia activating programmed cell death [16,19].

In regard to effect of age, gender and diabetes on the S100 protein expression in the CPN, this could be explained by the fact that Schwann cells which produce myelin have undergone death under the effect of metabolic disturbance through hyperglycemia and acceleration of the programmed cell death by the aging process, exaggerated by gender through the effect of the neurosteroidal hormones on Schwann cell function [18, 19, 20, 21].

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