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EDUCATION, RESEARCH AND HEALTH POLICY UNIT
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MEDICAL RESEARCH UNIT IN BIOCHEMISTRY.
DEPARTMENT OF NEUROLOGY AND NEUROPHYSIOLOGY.
FAMILY MEDICINE UNITS NUMBER 20, 41 AND 44, IMSS, CDMX.
SPECIALTY OF HUMAN ACUPUNCTURE OF THE ENMH OF THE IPN

Official Title of the study:

“Evaluation of the effect of electroacupuncture on the sensory symptoms of Symmetrical Distal Polyneuropathy of Diabetic origin and its correlation with changes in Nerve Conduction Velocity.”

MEXICAN SOCIAL SECURITY INSTITUTE AND EDUCATION,
RESEARCH AND HEALTH POLICY UNIT STUDY VERIFICATION ID:
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NCT Number: NOT AVAILABLE.

STATISTICAL ANALYSIS
DATE: August 24th, 2022.

A) CALCULATION OF THE SAMPLE SIZE

The calculation of the sample is based on research questions, this is on the effect of electroacupuncture and expected outcomes in relieving sensory symptoms, on changes in sensory and motor VCN and on the patient's quality of life. Therefore, the development of the sample size calculation will be for each outcome variable and in the end the calculations were concentrated in a summary table. We have suggested taking the highest value to be able to have the certainty of having sufficient statistical power and to be able to find all the differences of the proposed average comparisons.

Sample sizes:

1. For the third outcome, related to "relief". Will electroacupuncture be useful in relieving the sensory symptoms produced by PNDs of diabetic origin?

Table 1 of the review of Thakral G et al. (J Diabetes Sci Technol. 2013, 1;7(5):1202-9) was used as a reference, where different reports of the response and usefulness of electrical stimulation as a complementary treatment of painful and sensory diabetic neuropathy were analyzed and compared, according to this analysis the work of Dinesh Kumar et al. (Diabetes Care 1998, 21 (8): 1322-1325). Where we take into account the reported decrease in neuropathic symptoms with the use of electrical stimulation ($66 \pm 10\%$) when compared to a Sham ($55 \pm 12\%$). For the calculation of the N we use the comparison of means. ^{73,74}

	$n = K [(s_1)^2 + (s_2)^2] / (M_1 - M_2)^2$
K	14.884164 (Za+Zb) ²
s1	10 Standard deviation of group 1
s2	12 Standard deviation of group 2
m1	66 Group 1 Average
m2	55 Group 2 Average
n per group=	30
n total=	60
Za	2.576
Zb	1.282

For this research question a total of 60 subjects will be required, 30 for each group under study.

2. For the second outcome, will the use of electroacupuncture generate changes in the study of sensory and motor NCV by electrophysiological techniques?

For the question, it is calculated considering the mean and SD resulting at the beginning and end of the intervention of the changes of the NCV of Yanqing Tong et al. of 48.2 ± 0.6 vs 46.2 ± 0.7 (m / s).⁶¹ A comparison of means was made, with the test assumption of a bilateral hypothesis, with a Confidence or Security Level (1- α): 95%, Statistical Power of 80%, Accuracy (d) of 20%, the resulting sample size was:

196 subjects per group

3. For the third outcome, will the use of electroacupuncture generate improvement in the evaluation of the patient's quality of life as measured by the SF36?

So far there is no publication that analyzes the perception of an improvement in quality of life in subjects with diabetic neuropathy with the use of electroacupuncture. Therefore, we have raised the size according to those described in the article by *Kongkiat Kulkantrakorn et al.*⁷⁵ where the aforementioned authors made a "baseline" estimate of the perception of the quality of life of the patient with diabetic foot in 8 domains of the SF-36. Our proposal is that there will be an improvement in the quality of life, in all the domains described, that is, it will be at least equal or better, we have taken into account the average of the physical function which is the deteriorated of 18.9.

Table 4: Comparison of SF-36 mean score in relevant studies

Domains	Multiple sclerosis* (N=85)	Epilepsy* (N=271)	Diabetes* (N=555)	Diabetic foot ulcer [†] (Thailand) (N=40)	Healthy Thai adults [‡] (N=1,345)	PDN current study (N=33) [§]	Current study [§] VS Healthy Thai adults [‡] (P value)
Physical function	33.5	78.9	74.2	18.9	77.3±17.4	31.5±27.7	<0.001 [†]
Role limitation due to physical problem	32.7	60.0	55.1	27.5	82.2±28.6	40.2±39.0	<0.001 [†]
Bodily pain	72.2	72.8	71.7	32.5	75.6±18.4	47.3±30.6	<0.001 [†]
General health	53.7	68.7	52.6	79.5	65.1±18.1	50.8±28.5	0.007 [†]
Vitality	41.9	55.4	54.3	70.9	62.2±13.3	60.9±35.9	0.63
Social functioning	60.2	77.3	81.2	50.9	78.2±18.2	63.6±29.7	0.008 [†]
Role limitation due to emotional problem	59.6	66.3	70.2	51.6	80.4±31.9	42.4±43.5	<0.001 [†]
Emotional well being	67.8	68.4	73.0	36.6	66.1±12.9	39.4±30.7	<0.001 [†]

*Reference 22; [†]Reference 20; [‡]Reference 8; [§]Current study; [†]Statistically significant; PDN: Painful diabetic neuropathy; SF-36: Short form 36 quality of life questionnaires

Table taken from J Neurosci Rural Pract. 2013 Jul-Sep; 4(3): 267–270.⁷⁵

Making a comparison of averages:

$$n = K [(s1)^2 + (s2)^2] / (M1 - M2)^2$$

K	14.884164	(Za+Zb) ²
s1	7.37	Standard deviation of group 1
s2	27.7	Standard deviation of group 2
m1	31.5	Group 1 Average
m2	18.9	Group 2 Average
n per group=	77	
n total=	154	
Za	2.576	
Zb	1.282	

NOTE: As we discussed previously, we have suggested taking the highest value in order to be sure of having sufficient statistical power and to find all the differences in the proposed mean comparisons of each outcome (that is, the effect of Electroacupuncture compared to sham on the following outcome variables).

Each group was added 20% of the N for possible losses.

OUTCOME VARIABLE	N PER GROUP	ALPHA Z	Z DE BETA	PLUS 20% OF POSSIBLE LOSSES PER GROUP	STUDY TOTAL
Relief of sensory symptoms	30	2.576	1.282	36 Total :36	72
Changes in the study of sensory and motor VCN	109	2.576	1.282	130.8 Total: 131	262
Improvement in quality of life assessment	77	2.576	1.282	92.4 Total: 93	186

The sample of the present study was estimated at 109 for each group, however, 20% was added considering possible losses (each group will be 131), therefore, the total N of the study is 262.

It is important to mention that the way to obtain the sample will be by number of consecutive cases of candidate patients who go to the outpatient consultation of the UMF 20, 41 and 44 of the IMSS of the CDMX, for their subsequent evaluation in the screening and respective randomization in case of being included in the study.

VARIABLES

TABLE 1. CATEGORIZATION OF VARIABLES

	<i>VARIABLE</i>	<i>MEASURING INSTRUMENT</i>	<i>TYPE AND SCALE OF MEASUREMENT</i>	<i>UNITS</i>
<i>DEPENDENT</i>	Sensitive commitment	MDNS	Ordinal qualitative	Normal, diminished and absent
	Brawn	MDNS	Ordinal qualitative	Normal, moderate, severe and absent
	Reflexes	MDNS	Ordinal qualitative	Present, present with effort and absent.
	Sensory symptoms	MNSI	Discrete Quantitative	Dichotomous Response (Yes, No) Evaluation score 13 out of 15
	Aquilian reflex	MNSI	Ordinal qualitative	Present, present with effort and absent.
	Vibration perception	MNSI	Ordinal qualitative	Present, present with effort and absent.
	Pain scale	NRS	Discrete Quantitative	Evaluation score 0-10
	Physical function	SF 36	Ordinal qualitative	Excellent, very good, good, regular, bad
	Physical role	SF 36	Ordinal qualitative	Yes, it limits me a lot /Yes, it limits me a little/ No, it doesn't limit me at all.
	Body pain	SF 36	Ordinal qualitative	No, none/ Yes, very little/ Yes, a little/ Yes moderate/ Yes, a lot/ Yes, a lot
	General health	SF 36	Ordinal qualitative	Nothing, a little, Regular, quite, a lot.
	Vitality	SF 36	Ordinal qualitative	Always, almost always, sometimes, only once, never.
	Social function	SF 36	Ordinal qualitative	Always, almost always, sometimes, only once, never.
	Emotional role	SF 36	Ordinal qualitative	Always, almost always, sometimes, only once, never
	Mental health	SF 36	Ordinal qualitative	Totally true, Quite true, I don't know, Quite false, Totally false
	VCN	Electrophysiological techniques (Nicolet, Viking Model IV D, 4 channels)	Continuous quantitative	Latency (ms) Amplitude (mV) Distance (mm) Speed (m/s)
<i>INDEPENDENT</i>	Electroacupuntal points	Zusanli (E36)	Dichotomous qualitative	Dichotomous (Yes, No)
		Fenlong (E40)	Dichotomous qualitative	Dichotomous (Yes, No)
		Yinlingquan(B9)	Dichotomous qualitative	Dichotomous (Yes, No)
		Sanyinjiao (B6)	Dichotomous	Dichotomous

			qualitative	(Yes, No)
		Taichong (H3)	Dichotomous qualitative	Dichotomous (Yes, No)
		Zulinqi (VB41)	Dichotomous qualitative	Dichotomous (Yes, No)
CONFUSING	Age	Years	Discrete quantitative	40-70 years
	Fasting glyceimic control	mg/dl	Discrete Quantitative	100 -180 mg/dl
	Glycosylated hemoglobin concentration	Percentage	Continuous Quantitative	7-10 %
	DM evolution time	Years	Discrete quantitative	5-15 years of evolution
	BMI	Kg/m ²	Discrete quantitative	18-40 Kg/m ²
	Pressure	Mmhg	Discrete quantitative	90/60 mmHg-200/160 mmHg

STATISTICAL ANALYSIS

The collected variables will be integrated into a database in Excel and then analyzed in SPSS (Statistical Product and Service Solutions) for Windows in the 2019 version. The descriptive analysis of the information collected will be carried out using simple and absolute frequencies, as well as measures of central tendency and dispersion. Once the variables have been collected, the Shapiro-Wilk statistic will be applied to determine if their distribution is normal or not.

The statistical methods to be used will be two-tailed t-tests for comparison of mean value between groups, paired t-tests will be used for comparison of mean values within groups, and repeated two-way analysis of variance for changes in NCV, pain threshold and glucose concentrations.

To evaluate the degree of association between variables and covariates, univariate and bivariate models will be used using the X² test or Fisher's exact test for discrete variables; or the Student's t test, for continuous variables.

The analysis of these tests will be carried out by means of the Mann-Whitney U statistical test, or by means of ANOVA-type statistical measurement. Correlation studies will be performed by Pearson or Spearman Test.

A multivariate analysis will be carried out including the confounding variables (age, time of evolution of DM, anthropometric and biochemical parameters) using a non-conditional logistic regression model, for discrete dependent variables; and a linear regression model for continuous dependent variables.

The analysis of clinical improvement by means of the MDNS scale, the MNSI and the SF-36 will be performed by means of the Mann-Whitney U statistical test. The analysis of nerve conduction velocities shall be carried out by means of ANOVA-type statistical measurement.

It is contemplated to perform an analysis of multiple results and by subgroups, that is, by stratification according to age, time of diagnosis of DM, degree of affection, scale of pain and type of conventional pharmacological treatment, evaluating sensation and perception of the stimulus of pain, vibration, reflexes, VCN by electrophysiological techniques.

Although both groups may have improvement due to conventional treatment, significant differences or trends in the electroacupuncture group are expected, the analysis will include logistic regression analyzing the confounding variables.

For all tests a value of $p < 0.05$ shall be considered statistically significant.