Expression of Nerve Growth Factor (NGF) and its receptor (TrK A) in chronic renal failure patients with pruritus

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Abstract

Background: Chronic renal failure or chronic renal disease (CRD) is a progressive loss in renal function over a period of months or years. It is differentiated from acute kidney disease in that the reduction in kidney function must be present for over 3 months**.**

Uremic pruritus is the most common cutaneous abnormality in patients with CRF. Uraemic pruritus is unrelated to sex, age, duration, cause of dialysis, a list of unproven suggestions for pruritis include : dry skin; mast cell proliferation; Th1 cytokines; secondary hyperparathyroidism; disturbed balance between μ-opioid and κ-opioid receptors; substance P; increased skin ions Ca, Mg, PO; abnormal transmission via the spine .

Nerve growth factor is a neurotropic polypeptide necessary for the survival and growth of some central neurons, as well as sensory afferent and sympathetic neurons**.**

It has been found that in AD patients increased levels of NGF in keratinocytes and infiltrating leukocytes which were related to disease severity **.** Skin from patients with prurtigo nodularis also have increased presence of NGF which is primarily found in infiltrating leukocytes.

Method: The present case-control study was carried out on 30 patients with CRF and uraemic pruritus on hemodialysis who were treated at Sohag Educational Hospital and 20 healthy control subjects. Patients with CRF and uremic pruritus on hemodialysis of both sex, any age and not received any systemic treatment for pruritus for at least 3 weeks prior to the study were included in this study.

Severity of itching was measured by the 5-D itch score. 5-D is (duration – degree – direction –disability and distribution). Maximum score is 25 and indicate severe itching while the minimum score is 5 and indicate no itching**.** Skin punch biopsies (3 mm) were taken from them for immunohistochemical studies**.**

Results: The present study found that in the skin of the healthy control participants the expressions of NGF were seen in the epidermal basal layer, but there was no expression in upper and mid epidermal cell layers while in dermis about 75% of them showed mild expression and 25% showed moderate expression.

In patients with chronic CRF with pruritus on dialysis the expressions of NGF were strong in epidermal basal layers, upper and mid epidermal layers. There was a strong expression of NGF in dermis with some spindle like infiltrating cells especially in dermal papillae.

The present study found that in the skin of the healthy control participants the expression of TrKA were seen in the epidermal basal layer, but there was no

expression in upper and mid epidermal cell layers while in dermis 75% of them showed mild expression while 25% showed moderate expression.
In patients with chronic CRF with pruritus on dialysis the expressions of TrKA were strong in epidermal basal layers, mid and upper epidermal layers. In dermis especially upper dermis larger number of cells demonstrated strong TrkA immunoreactivity.
This study found that there was a significant correlation between the 5-D itch score and age of the patients. There was a significant correlation between the 5-D itch score and degree of expression of both NGF and TrKA.
Conclusion: A significant increase in the expressions of NGF and TrKA in CRF patients with pruritus on hemodialysis compared to the healthy control participants and also there was significant correlation between degree of expressions of NGF and TrKA and 5-D itching score could be the cause of initiation and maintenance of pruritus in CRF patients with pruritus on dialysis.

Introduction

Chronic renal failure or chronic renal disease (CRD), is a progressive loss in renal function over a period of months or years. It is differentiated from acute kidney disease in that the reduction in kidney function must be present for over 3 months **(1).** In Egypt; end stage renal disease (ESRD) is growing by 100% annually; the estimated annual incidence of ESRD is around 74 per million and the total prevalence of patients on dialysis is 264 per million, also there are 90,000 patient die each year because of kidney failure **(2).** In the El-Minia governorate, one of the upper Egypt governorates, the prevalence was 308 per million **(3).**
The main causes for CRD are diabetes, hypertension, glomerulonephritis, and polycystic kidney disease **(4)**.
The major skin manifestations in CRD are xerosis, pruritus, hyperpigmentation, perforating and calcifying disorders and bullous diseases **(5, 6).**
Uraemic pruritus (UP) or renal itching is a common and distressing problem for people with CRD. When severe, it leads to sleep deprivation, depression and is associated with an increased risk of death. **(7).** In Egyptian patients with CRF on hemodialysis the prevalence of pruritis was 55% **(8)**.
Uraemic Pruritus is unrelated to sex, age, duration, cause of dialysis, a list of unproven suggestions for pruritis include : dry skin; mast cell proliferation; Th1 cytokines; secondary hyperparathyroidism; disturbed balance between μ-opioid and κ-opioid receptors; substance P; increased skin ions Ca, Mg, PO; abnormal transmission via the spine (**9**).
Nerve growth factor is a neurotropic polypeptide necessary for the survival and growth of some central neurons, as well as sensory afferent and sympathetic neurons **(10).** It elicits a number of biologic effects on non-neural cells of the immune-inflammatory complement and it is believed that NGF might also play role in inflammatory, autoimmune and allergic disorders **(11).** Antidromic C (conducting impulses in a direction opposite to the normal, said of neurons of the posterior roots of the spinal cord) nerve signaling from CNS to the skin may cause the release of neuropeptides from the peripheral nerve endings that can induce a cascade of proinflammatory events **(12, 13).**

Patients and Methods :

The present case-control study was carried out on 30 patients with CRF and uraemic pruritus on hemodialysis who were treated at Sohag Educational Hospital and 20 healthy control subjects.

Patients with CRF and uremic pruritus on hemodialysis of both sex, any age and not received any systemic treatment for pruritus for at least 3 weeks prior to the study were included in this study. Patients with history suggestive of other medical illness as diabetes mellitus, liver disease and thyroid function abnormalities and primary skin diseases were excluded.

All patients underwent complete history taking included; 1) Personal history as; age, sex, marital status, residence, occupation and special habits of medical importance as smoking. 2) Medical history as; onset, course and duration of renal disease and current treatment. All patients were subjected to clinical general examination and local dermatological examination included skin, mucous membranes, hair and nail.

Severity of itching was measured by the 5-D itch score **(14)**, which was done by a single paper questionnaire that not only measure the intensity of itching but also its impact on patient quality of life, it also detect its change over time. 5-D is (duration – degree – direction –disability and distribution). Maximum score is 25 and indicate severe itching while the minimum score is 5 and indicate no itching**.**

The scores of each of the five domains are achieved separately and then summed together to obtain a total 5-D score. 5-D scores can potentially range between 5 (no pruritus) and 25 (most severe pruritus).

Material:

Buffer: TBS buffer (Tris HCL 6.19g, NaCl 8.8g, Aq.dest. 1000 ml, PH 7.6), (TBS + 3.5 % H2O2), Posphate buffer solution (PBS) PH 6.

Antibodies: Primary antibodies: A rabbit polyclonal IgG anti-human NGF antibody (Sant Cruz biotech). A rabbit polyclonal IgG anti-human TrKA antibody (Sant Cruz biotech). Secondery antibodies: Biontinylated goat anti-rabbit antibody.

Method:

Skin specimen:

Skin punch biopsy samples were taken from patients and controls. The sizes of biopsies were 5 mm which were taken under local anesthesia (lidocaine 0.5%). The samples were fixed in formalin and processed for paraffin section. These samples were treated and processed parallel with control healthy human samples which already were taken from healthy volunteers.

Staining method:

The biopsies were processed for immunohistochemical studies. For this purpose vertical paraffin section (7mm) were prepared and stored for immunohistochemical staining procedures.

Parrafin section were deparaffinized and hydrated then they were washed in TBS buffer for further hydration. Subsequently, the slides were washed in TBS + 3.5 % H2O2 and then again they were washed in TBS buffer. Antigen retrieval was done using trypsin, then sections were incubated in protein blocking agent.

The intensity of staining was designated as:

(-): Negative staining (no positive cells). (+): Mild intensity of staining. (++): Moderate intensity of staining. (+++): Strong intensity of staining.

Statistical analysis: Data was analyzed using STATA intercooled version 12.1. Quantitative data was represented as mean, standard deviation, median and range. T test was used to compare two groups. Test for trend was used to compare ordered groups. Pearson's correlation analysis was used to find the association between age and 5-D itching score. Univariate and multivariate logistic regression was used to determine factors affect 5-D itching score. Graphs were produced by using Excel or STATA program. P value was considered significant if it was less than 0.05.

Results

This study included 30 CRF patients with pruritus on haemodialysis and 20 healthy controlled subjects.

In stratum basale, about 75% (n=15) of control samples showed mild expression of NGF, while about 25% (n=5) showed moderate expression of NGF. By contrast about 20% (n= 6) of chronic renal failure cases showed mild expression of NGF, 36.67% (n=11) showed moderate expression of NGF and 43.33% (n=13) showed marked expression of NGF.

Table (1) Comparison between control and CRF groups according to expression of NGF in stratum basale.

Stratum basale	Expression	Control	CRF patients	P value
	Mild	15 (75%)	6 (20.00%)	<0.0001
	Moderate	5 (25.00%)	11 (36.67%)	
	Strong	0	13 (43.33%)	

CRF: chronic renal failure. **NGF:** nerve growth factor. **P value** < 0.05 was significant

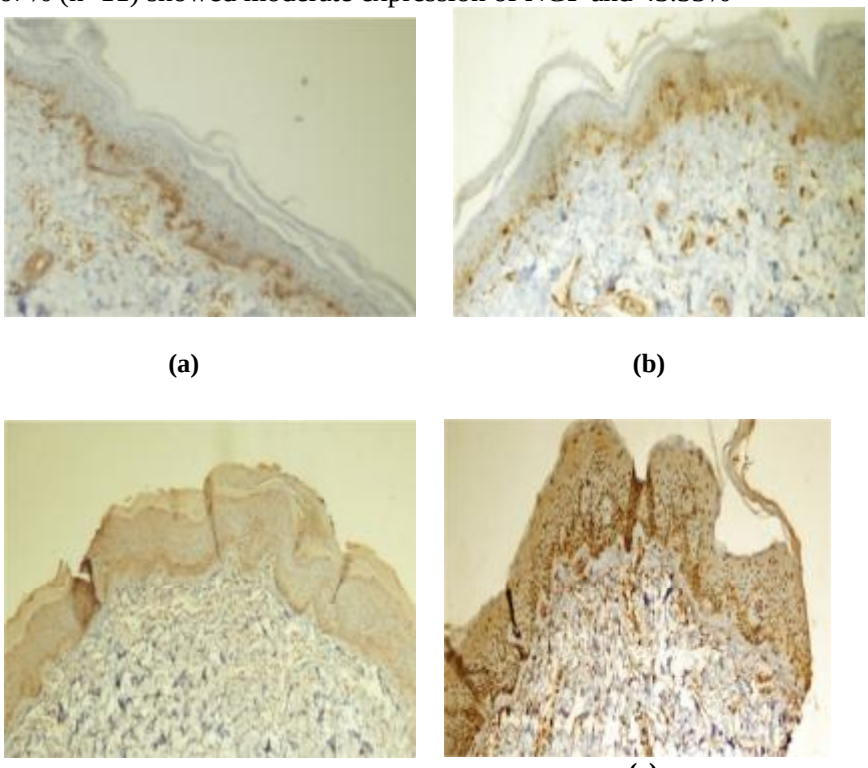
In mid epidermis, all control samples 100% (n=20) showed no expression of NGF in mid epidermis, By contrast about 20% (n=6) of CRF patients showed mild expression of NGF, 36.67% (n=11) showed moderate expression of NGF and 43.33% (n=13) showed marked expression of NGF.

Table (2) Comparison between control and CRF groups according to expression of NGF in mid epidermis.

Mid epidermis	Expression	Control	CRF patients	P value
	Negative	20 (100%)	6 (20.00%)	<0.0001
	Moderate	0	11 (36.67%)	
	Strong	0	13 (43.33%)	

CRF: chronic renal failure. **NGF:** nerve growth factor. **P value** < 0.05 was significant

In upper epidermis, all control samples 100% (n=20) showed no expression of NGF in mid epidermis. By contrast about 20% (n=6) of CRF patients showed mild expression of NGF, 36.67% (n=11) showed moderate expression of NGF and 43.33%



(a) **(b)**

(c)

(d)

Figure (1) NGF expressions in the epidermis:

(a) Expressions of NGF in control. (b) Weak expressions of NGF in CRF patients. (c) Moderate expressions of NGF in CRF patients. (d) Strong expressions of NGF in CRF patients.

Table (3) Comparison between control and CRF groups according to expression of NGF in upper epidermis.

Upper epidermis	Expression	Control	CRF patients	P value
	Negative	20 (100%)	6 (20.00%)	
	Moderate	0	11 (36.67%)	
	Strong	0	13 (43.33%)	<0.0001

CRF: chronic renal failure. **NGF:** nerve growth factor. **P value** < 0.05 was significant

In dermis, about 75% (n=15) of control samples showed mild expression, while 25% (n=5) showed moderate expression of NGF. By contrast about 13.33% (n=4) of CRF patients showed mid expression, 43.33% (n=13) showed moderate expression and 43.33% (n=13) showed marked expression of NGF .

Table (4) Comparison between control and CRF groups according to expression of NGF in dermis.

Dermis	Expression	Control	CRF patients	P value
	Mild	15 (75%)	4 (13.33%)	<0.0001
	Moderate	5 (25.00%)	13 (43.33%)	
	Strong	0	13 (43.33%)	

CRF: chronic renal failure**. NGF:** nerve growth factor. **P value** < 0.05 was significant.

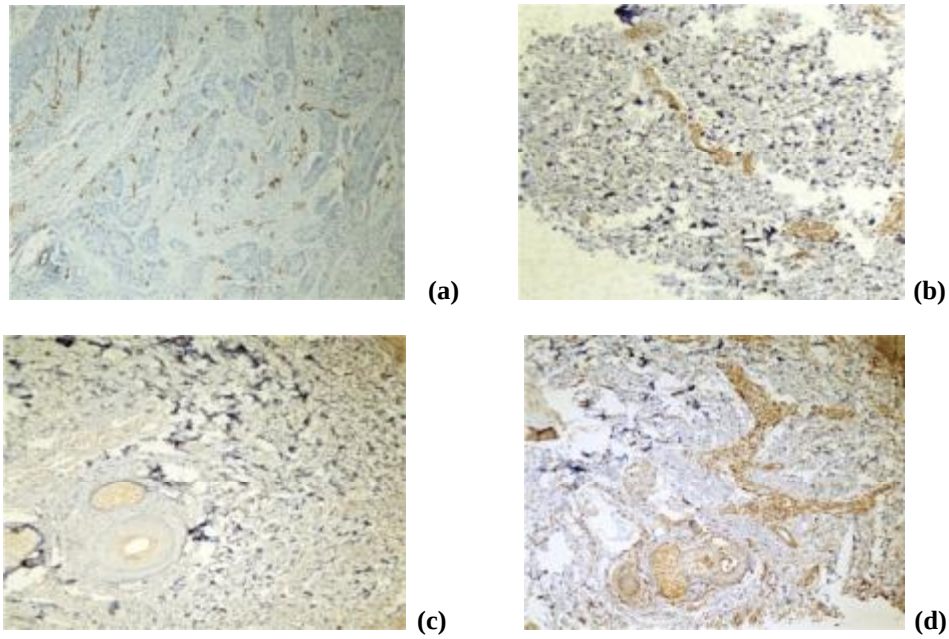


Figure (2) Expressions of NGF in the dermis:
(a) NGF expressions in control. (b) Mild expressions of NGF in CRF patients. (c) Moderate expressions of NGF in CRF patients. (d) Strong expressions of NGF in CRF patients.

In stratum basale, about 75% (n=15) of control samples showed mild expression, while 25% (n=5) showed moderate expression of TrKA. By contrast 23.33% (n=7) of CRF patients showed mild expression, 33.33% (n=10) showed moderate expression and 43.33% (n=13) showed strong expression of TrKA.

Table (5) Comparison between control and CRF groups according to expression of TrKA in Stratum basale.

Stratum basale	Expression	Control	CRF patients	P value
	Mild	15 (75%)	7 (23.33%)	<0.0001
	Moderate	5 (25.00%)	10 (33.33%)	
	Strong	0	13 (43.33%)	

NGF: nerve growth factor. **TrKA**: tyropomyosin kinase. **P value** < 0.05 was significant.
In mid epidermis, all control samples 100% (n=20) showed no expression of TrKA. By contrast, about 20% (n=6) of CRF patients showed mild expression, 36.67% (n=11) showed moderate expression and 43.33% (n=13) showed strong expression of TrKA .

Table (6) Comparison between control and CRF groups according to expression of TrKA in mid epidermis.

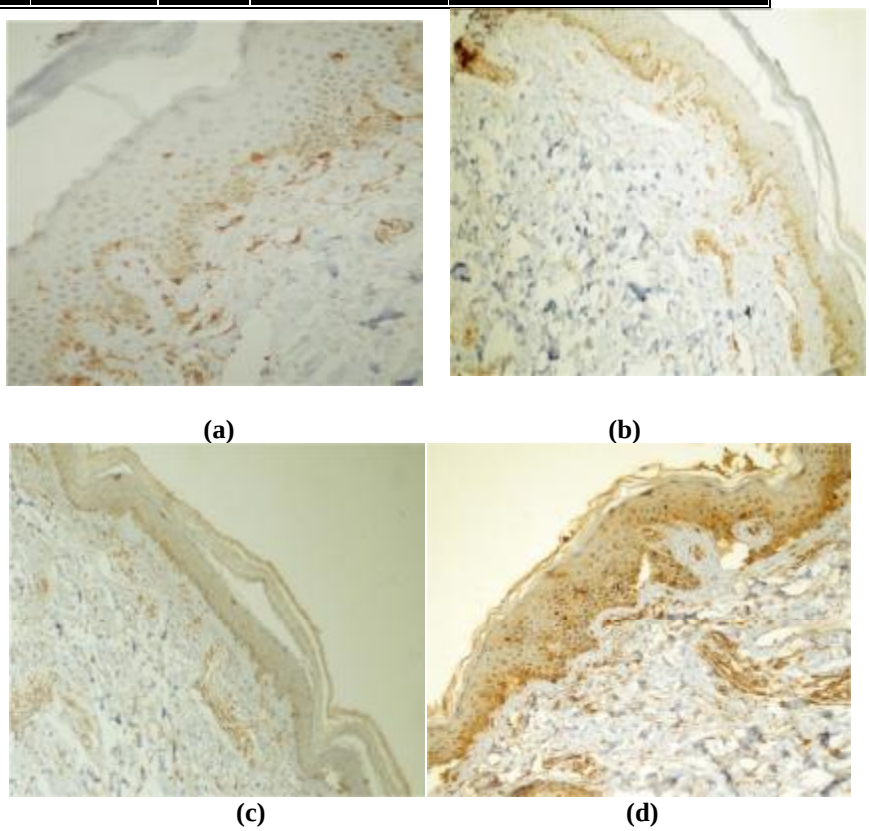
Mid epidermis	Expression	Control	CRF patients	P value
	Negative	20 (100%)	6 (20.00%)	<0.0001
	Moderate	0	11 (36.67%)	
	Strong	0	13 (43.33%)	

NGF: nerve growth factor. **TrKA:** tyropomyosin kinase. **P value** < 0.05 was significant.

In upper epidermis, control samples 100% (n=20) showed no expression of TrKA. By contrast, about 20% (n=6) of CRF patients showed mild expression, 40% (n=12) showed moderate expression and 40% (n=12) showed strong expression of TrKA.

Table (7) Comparison between control and CRF groups according to expression of TrKA in upper epidermis.

Upper epidermis			Expression	Control	CRF patients	P value
			Negative	20 (100%)	6 (20.00%)	<0.0001 **P value** < 0.05 was significant.
			Moderate	0	12 (40.00%)	
	Strong	0	12 (40.00%)			



(a) **(b)**

(c) **(d)**

Figure (3) Expressions of TrKA in epidermis:

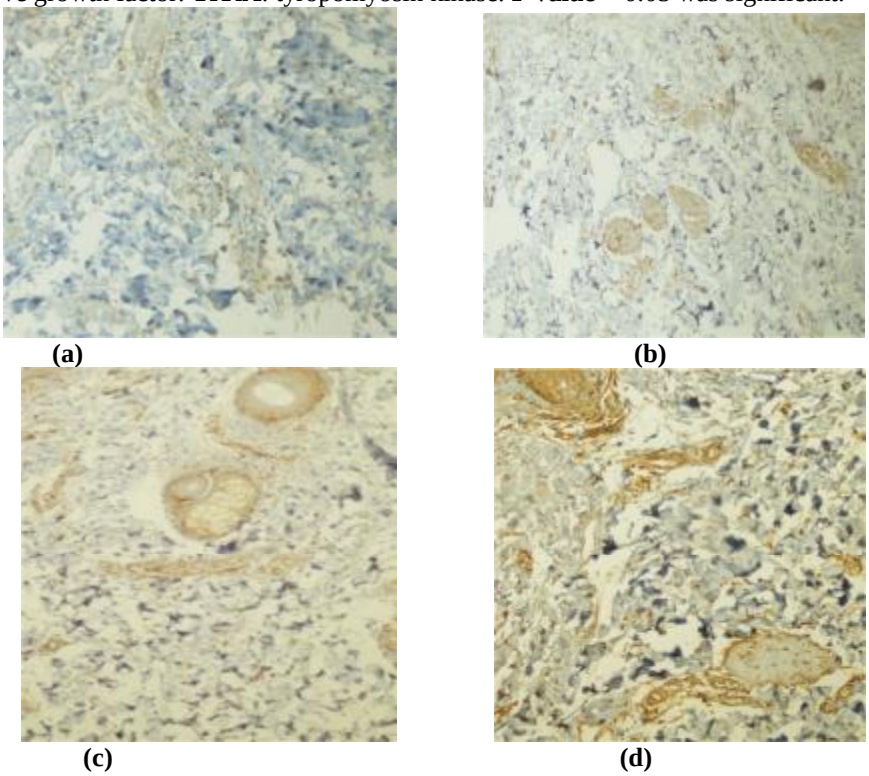
(a) TrKA expressions in control. (b) Mild TrKA expressions in CRF patients.
(c) Moderate TrKA expressions in CRF patients. (d) Strong TrKA expression in CRF patients.

In dermis, about 75% (n=15) of control samples showed mild expression, while 25% (n=5) showed moderate expression of TrKA. By contrast 13.33% (n= 4) of CRF patients showed mild expression, 43.33% (n=13) showed moderate expression and, 43.33% (n=13) showed strong expression of TrKA.

Table (8) Comparison between control and CRF groups according to expression of TrKA in dermis.

Dermis	Expression	Control	CRF patients	P value
	Mild	15 (75%)	4 (13.33%)	<0.0001
	Moderate	5 (25.00%)	13 (43.33%)	
	Strong	0	13 (43.33%)	

NGF: nerve growth factor. **TrKA**: tyropomyosin kinase. **P value** < 0.05 was significant.



(a) **(b)** **(c)** **(d)**

Figure (4) Expressions of TrKA in dermis:
(a) Expressions of TrKA in control. (b) Mild expressions of TrKA in CRF patients. (c) Moderate expressions of TrKA in CRF patients.(d) Strong expressions of TrKA in CRF patients.

In CRF patients, the mean (SD) of 5-D itch score was 17.33 (4.4) and median (range) 17 (10-24).

Table (9) Distribution of 5-D itching score among studied population in CRF patients.

5-D itching score	Data
Mean (SD) Median (range)	17.33 (4.4) 17 (10-24)

CRF: chronic renal failure **SD:** standard deviation

There was a significant correlation between degree of expression of NGF and 5-D itching score. In weak expression of NGF, the 5-D itch score mean ± SD was 10.83 (0.98) and the median (range) was 10.5 (10-12). In moderate expression of NGF the 5-D itch score mean ± SD was 15.73 (1.01) and the median (range) was 16 (14-17). In strong expression of NGF the 5-D itch score mean ± SD was 21.69 (1.54) and the median (range) was 22 (19-24)**.**

Table (10) Relation between expression of NGF and 5-D itching score.

Expression of NGF	5-D itching score Mean ± SD Median (range)	P value
Weak	10.83 (0.98) 10.5 (10-12)	<0.0001
Moderate	15.73 (1.01) 16 (14-17)	
Strong	21.69 (1.54) 22 (19-24)	

P value <0.05 was significant. **SD:** standard deviation

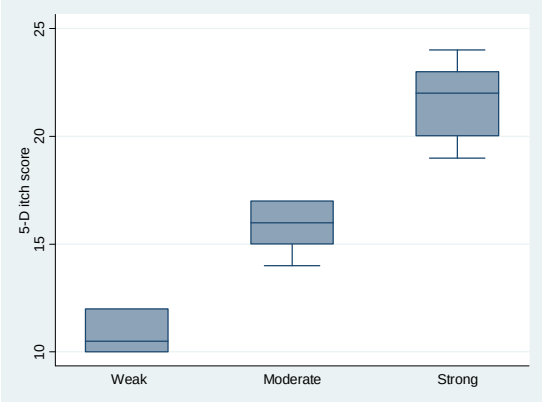


Figure (5) Relation between expression of NGF and 5-D itching score.

There was a significant correlation between degree of expression of TrKA and the 5-D itching score. In weak expression of TrKA; mean ± SD was 10.83 (0.98) and the median (range) was 10.5 (10-12). In moderate expression of TrKA; mean ± SD was 15.73 (1.01) and the median (range) was 16 (14-17). In strong expression of TrKA; mean ± SD was 21.69 (1.54) and the median (range) was 22 (19-24) **.**

Table (11) Relation between expression of TrKA and 5-D itching score.

Expression of TrKA	5-D itching score Mean ± SD Median (range)	P value
Weak	10.83 (0.98) 10.5 (10-12)	<0.0001
Moderate	15.73 (1.01) 16 (14-17)	
Strong	21.69 (1.54) 22 (19-24)	

TrKA: tyropomyosin kinase. **SD:** standard deviation**.**

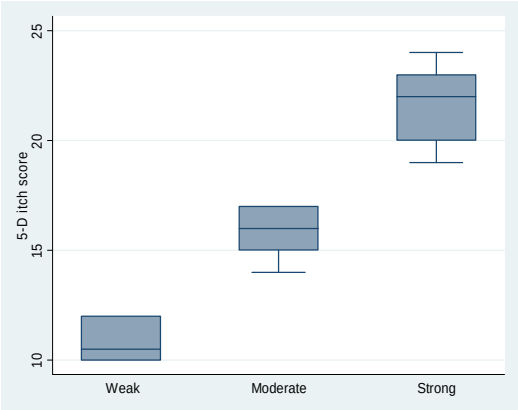


Figure (6) Relation between expression of TrKA and 5-D itching score.

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