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Effect of miR-146a polymorphism on lipoic acid therapy in patients with T2DM peripheral polyneuropthy

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ABSTRACT

For investigating the impact of miR-146a rs2910164 polymorphism on the therapeutic efficacy of lipoic acid therapy in patients with type 2 diabetes mellitus (T2DM) peripheral neuropathy (DPN). 106 T2DM-DPN patients in our hospital from Jan. 2020- 2022 were selected. The probe detection method was utilized to determine the polymorphism of the miR-146a rs2910164 gene in peripheral blood. All patients were treated with zinc sulfate for 3 weeks period. According to the treatment effect, 37 patients who were ineffective in treatment will be divided into an ineffective group, and 79 patients who were effective in treatment will be divided into an effective group. The condition of miR-146a gene peptides was analyzed after treatment in both groups. The motor nerve conduction velocity (MNCV), sensory nerve conduction velocity (SNCV), and Toronto Clinical Scoring System (TCSS) scores of the median nerve and common peroneal nerve with different genotypes were compared between the 2 sets. The genotype frequencies of alleles G, GG, and GC in the valid group were lower than those in the invalid group; After treatment, MNCV and SNCV of CC genotype median nerve and common peroneal nerve in DPN patients were higher than those before treatment; The TCSS scores of the three genotypes less than post-treatment. The above results showed statistically significant differences ($P<0.05$). Lipoic acid is influenced by the miR-146a polymorphism gene in the treatment of T2DM-DPN patients, with the CC genotype having a lower susceptibility and the best clinical treatment effect.

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Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disease represented by chronic high blood glucose. With the improvement of China's economic level and living standard, the disease is increasing year by year (1). Diabetic Peripheral Neurophathy (DPN) is a chronic complication with the highest incidence rate and complexity in T2DM. Investigation shows that the incidence rate of DPN in T2DM patients can reach 42.2%. Mild symptoms can cause abnormal changes in neural structure, leading to limb numbness and sensory decline; Severe patients may develop foot ulcers and gangrene after the condition worsens, ultimately leading to limb disability (2). Currently, lipoic acid is the main drug treatment method for DPN in T2DM patients, but there are certain differences and limitations in the treatment effect due to the influence of relevant factors (3). miR-146a belongs to an RNA multifunctional non-coding small molecule, derived from microRNA (miRNA), which exists in inflammatory reactions and is closely related to them. miR-146a can act on tumor necrosis receptor-related factor 6 (TRAF-6) and Interleukin-1 receptor-associated kinase 1 (IRAK-1), affecting the NF-κB pathway through regulation and occurs a vital position in producing and developing DPN (4). miR-146 is a factor involved in regulating the natural immune system in the body. It has an impact on cell metabolism through regulatory effects, especially on the inflammatory response process of the body, and can also affect the treatment process of diseases. Compared to healthy individuals, the miR-146a level in the peripheral blood of T2DM patients is relatively low. The main reason for its low level is that T2DM patients have significant susceptibility, and there are different differences in the treatment effects of zinc sulfate among DPN patients with different miR-146a genotypes. This research topic provides important evidence for clinical disease treatment by analyzing the susceptibility of the miR-146a gene's peptide polymorphism in the treatment of DPN patients with zinc sulfate. The research content is reported as follows.

Materials and Methods

General Data

106 T2DM-DPN patients who were treated in our hospital from Jan. 2020 to Jan. 2022 were chosen for investigation and data collection. Both groups were treated with zinc sulfate, and after 3 weeks of treatment, patients were separated into groups grounded on their therapeutic effects. The ineffective treatment group consisted of 37 cases; The effective treatment group consists of 79 cases. Comparative information includes two groups: gender, age, T2DM course, course of DPN, BMI, Fasting plasma glucose (FPG), Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Triglyceride (TG), Glycosylated hemoglobin (HbA1c), Low-density lipoprotein (LDL), Human total cholesterol (TC), High-density lipoprotein (HDL) and Insulin resistance index (HOMA-IR). There was a statistically insignificant difference in the comparison of the above data ($P>0.05$) (Table 1).

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Inclusive criteria & Exclusive criteria

Inclusion criteria

(A) Clinical symptoms (overeating, drinking, and urination) were evaluated. Patients with FPGof more than 7.0 mmol/L, random blood glucose of more than 11.1 mmol/L, or HbA1c of more than 6.5% were diagnosed as diabetes, and the course of the disease was more than 1 year; (B) Comply with the diagnostic criteria related to DPN in the 2017 Diabetes Prevention and Control Guide (5); (C) Peripheral neuropathy occurs when or after the diagnosis of diabetes; (D) There are obvious symptoms of limb pain, numbness, and sensory abnormalities.

Exclusion criteria

(A) Neuroconductive dysfunction caused by long-term medication or alcohol consumption; (B) Peripheral neuropathy caused by osteoarthritis, cervical and lumbar spine diseases, and stroke; (C) Patients with lymphangitis or venous thrombosis; (D) Severe dysfunction of the heart, lungs, liver, and kidneys in the body; (E) Patients with bone metabolism abnormalities, immune system diseases, or malignant tumors.

Research Methods

Treatment method

All patients were treated with blood sugar control and zinc sulfate medication. Blood sugar treatment control: to give medical advice. Patients follow the advice to apply hypoglycemic drugs or monitor blood sugar levels through insulin injection. The patient needs to maintain a FPG level of 4.0~7.6mmol/L, and a blood glucose level of 7.0~10.0mmol/L at 2 hours after meals. Lipoic acid medication: Zinc sulfate 600mg (Manufacturer: Yantai Zhichu Pharmaceutical Co., Ltd.; National drug approval number: H20080523; Specification: 300mg/piece), once daily for 3 weeks. After three weeks of treatment, the therapeutic effect was evaluated.

Grouping method

According to the DPN diagnostic and treatment standards, the advanced electromechanical evoked potential system is adopted to comprehensively evaluate the effectiveness of drug treatment by evaluating the limb nerve conduction velocity of patients' pre/post-treatment. Significant effect: The clinical symptoms of the peripheral nerves in the limbs almost disappear, and the tendon reflex shows a normal state. The SNCV and MNCV have increased by ≥5.0m/s compared to the pre-treatment velocity level, or have already reached normal human velocity levels. Effective: There are basically no clinical symptoms of peripheral nerves, and there is a significant improvement in tendon reflexes. The improvement of SCV and MCV is within the range of 3.0-5.0m/s compared to before treatment. Invalid: All symptoms are improved. Total effective rate= (significant+effective)/total number×100%. The final patient will be divided: effective; ineffective.

Observation indicators and evaluation criteria

General information

Based on the research content, the investigator made a self-made general data collection form for evaluation. The collected content mainly includes gender, age, T2DM period, FPG, TC, TG, DPN course, BMI, blood pressure, HbA1c, HDL, LDL, and HOMA-IR.

Laboratory index evaluation

The patient was instructed to take 5mL of fasting venous blood (FVB) in the morning and place it in a common blood collection vessel, keep it at room temperature for 30min, and centrifugate it for 15min with a high-speed cryogenic centrifuge at a speed of 3500 rpm after it was coagulated. Obtain serum for testing and application.

FPG, TC, TG, HDL, LDL

These are all evaluated using fully automated biochemical analysis instruments. HbA1c: In addition, the patient was instructed to extract 2mL of fasting venous blood in the morning and place it in the anticoagulant tube for detection by high-performance liquid chromatography. HOMA-IR: It first uses serum to detect Fasting serum insulin (FINS) values through a fully automated biochemical chemiluminescence immunoassay analyzer, and then uses the formula HOMA-IR=FPG×FINS/22.5 for calculation (6).

miR-146a polymorphism assessment

The patient needs to take 1mL of FVB in the morning and place it in an EDTA anticoagulant tube. DNA is extracted using the whole genome DNA kit of the blood. To select the miR-146a gene probe sequence and primers. The upstream primer in the miR-146a gene is 5'-GAACTGAATTCCATGGGTTTGT-3', and the downstream primer is 5'-GCCCACGAT-GACAGAGATCC-3'; Gene probe sequence: 5'-FAM-TCAGACCTGAAATT-MGB-3' is the specific sequence of probe G allele, and 5'-HEX-TCAGACCTCTGAAATT-MGB-3' is the specific sequence of probe C allele. The 7500 real-time polymerase chain reaction (PCR) system is applied to detect specific gene types and evaluate the amplification of target fragments (7). The specific conditions for the system reaction are: preheating temperature of 50℃ and time of 2 minutes; Observing the pre-denaturation in an environment with a temperature of 95℃ for 10 min; Performing denaturation at the same temperature for 15s; Reducing the temperature to 60℃ and anneal for the 40s, continuously implementing a total of 55 cycles. TaqMan classification uses a double-blind method for evaluation and judgment.

Clinical Symptom Scoring

Evaluated by investigators using the Toronto Clinical Scoring System (TCSS). The system consists of three parts, including neural reflex, neural symptoms, and sensory function (8). (I) Neural reflex: Knee reflex and ankle reflex, both lower limbs are normal, with a score of 0; (II) Neural symptom assessment: Symptom items include pain, limb numbness, fatigue, needle-like sensation, and similar upper limb symptoms, all of which are normal and scored 0 points, while those with symptoms are scored 1 point; (III) Sensory function evaluation: Symptom items include right toe pain, tactile pressure, temperature, position, and vibration, with 1-point for abnormal states and 0 for normal states. The total score of the system is 0-19 points, and the lower the score, the lighter the patient's symptoms.

Statistical analysis of data

SPSS26.0 was used to test the data, and the number of cases (n) and rate (%) were used to describe the counting data. x^2-tests were performed between groups. The mean ± standard deviation ($\bar{x} \pm s$) is used to describe the measurement data (normal distribution), and the independent

samples are compared before and after treatment by t-test. P<0.05 indicates a statistically meaningful distinction.

Results

Comparison of baseline data information between two groups

There was a statistically meaningless distinction in gender, age, duration of T2DM, duration of DPN, BMI, blood pressure, FPG, HbA1c, TC, TG, HDL-C, LDL-C, and HOMA-IR between the2 sets (P>0.05) (Table 1).

Comparison of genotype and allele frequency between two groups

The genotype frequencies of alleles G and GG in effective less than those in ineffective; The frequency of alleles C and CC in effective was higher. The difference in conclusions between them was statistically meaningful(P<0.05) (Table 2).

Comparison of MNCV and SNCV levels in DPN patients with different miR-146a genotypes before and after treatment

There was no significant difference in MNCV and SNCV of GG and GC genotypes of the median nerve and common peroneal nerve in DPN patients on pre and post-treatment (P>0.05). After treatment, the levels of MNCV and SNCV of CC genotype median nerve and common peroneal nerve were greatly higher than that post-treatment (P<0.05) (Table 3 and Figure 1).

Table 1. Comparison of baseline data information ($\bar{x} \pm s$, %).

Division	n	gender		Age of years	The course of T2DM (year)	The course of DPN (year)
		male	female			
ineffective group	37	20	17	54.31±5.67	8.42±2.16	1.67±0.27
effective group	69	30	39	54.61±5.08	8.53±2.09	1.71±0.19
x^2/t		1.081		0.278	0.255	0.888
P		0.298		0.781	0.799	0.376

Division	n	BMI (kg/m²)	Blood pressure (mmHg)		FPG (mmol/L)	HbA1c (%)
			SBP	DBP		
ineffective group	37	21.67±2.24	132.51±12.08	76.89±6.48	9.47±2.06	10.34±1.09
effective group	69	21.83±2.10	132.73±11.98	77.01±5.29	9.58±2.11	10.48±1.02
x^2/t		0.365	0.090	0.103	0.258	0.658
P		0.716	0.929	0.918	0.797	0.512

Division	n	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	HOMA-IR
Control group	37	4.75±1.13	1.59±0.24	1.20±0.26	2.64±0.57	3.29±1.64
Experimental group	69	4.93±1.05	1.60±0.07	1.24±0.19	2.77±0.49	3.57±1.57
x^2/t		0.819	0.043	0.905	1.229	0.862
P		0.415	0.966	0.367	0.222	0.391

Table 2. Comparison of genotype and allele frequency [n (%)].

Division	n	Genotype			Allele (n=128)	
		GG	GC	CC	G	C
ineffective group	37	19 (51.35)	16 (43.24)	2 (5.41)	53 (80.30)	13 (19.70)
effective group	69	21 (30.43)	12 (17.39)	36 (52.17)	53 (36.30)	93 (63.70)
*x*2		4.485	8.281	22.907	27.353	53.041
P		0.034	0.004	0.000	0.000	0.000

Table 3. The comparison of MNCV and SNCV levels in DPN patients with different miR-146a genotypes ($\bar{x} \pm s$).

GenotypemiR-146a	n	MNCV (m/s)			
		Nervus medianus		Nervus peroneus communis	
		before treatment	after treatment	before treatment	after treatment
GG	40	38.94±3.78	39.27±3.99#	34.98±3.19	35.28±4.00#
GC	38	38.51±3.39	40.05±4.11#	33.48±4.06	35.01±3.67#
CC	38	37.76±4.06	44.69±4.72*	34.76±4.38	40.87±3.56*
GenotypemiR-146a	n	SNCV (m/s)			
		Nervus medianus		Nervus peroneus communis	
		before treatment	after treatment	before treatment	after treatment
GG	40	32.24±3.16	33.45±3.20#	36.06±3.24	36.29±3.07#
GC	38	33.40±3.09	34.09±4.15#	36.27±3.51	37.06±3.61#
CC	38	32.18±3.75	42.57±4.71*	34.72±2.67	40.88±3.82*

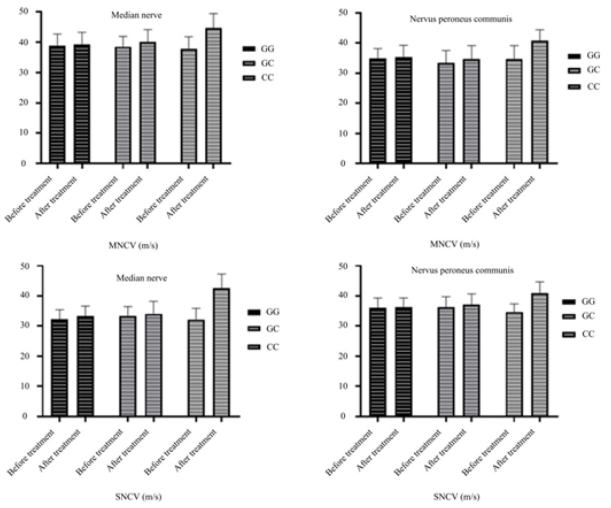

Figure 1. Comparison of MNCV and SNCV levels in DPN patients with different miR-146a genotypes before and after treatment.

Comparison of DPN patients with different miR-146a genotypes

The TCSS scores of GG, GC, and CC genotypes of pre-treatment were greatly lower($P<0.05$) (Table 4).

Discussion

The incidence rate of chronic complications in T2DM patients increases with the prolongation of the course of the disease. Long-term high blood sugar can cause a disorder of the body's supply system, lead to the metabolic disorder of peripheral nerve cells, and ultimately lead to the occurrence of DPN. Clinical studies have shown that the pathogenesis of DPN is very complex and occurs under the combined action of multiple factors. Genetic susceptibility factors are an important basis for its occurrence, and the disorder of glucose and lipid metabolism in the body activates the inherent immune mechanism, leading to chronic inflammation. During the entire process of damage to surrounding neurons, a large amount of oxygen free radicals are produced in local tissues, which not only leads to oxidative damage to neurons but also can downstream NF-κB mediates inflammatory damage (9). The interaction between oxidative stress and inflammatory response states in the body plays a joint role in nerve cell damage. As a signal pathway hub, NF-κB mediates oxidative stress and inflammatory response and is an important central link in the occurrence of DPN in diabetes (10). The clinical treatment plan for T2DM complicated with DPN is early detection, early treatment, and improvement of clinical symptoms to delay the condition. However, the early symptoms of complications are not significant and difficult to detect, making early clinical diagnosis more difficult. Therefore, how to screen high-risk patients with T2DM complicated by DPN and diagnose and implement treatment as soon as possible is an urgent focus for clinical attention (11). Zinc sulfate is a strong antioxidant. When the drug acts on the body, it can effectively eliminate oxygen free radicals in cells and promote the restoration of blood flow on the neurointimal surface to a normal state. Meanwhile, zinc sulfate can improve nerve conduction constraints, enhance the body's antioxidant capacity, and avoid oxidative stress damage caused by lesions, making it an important means of clinical treatment for DPN (12). The mechanism of action of clinical zinc sulfate in treating DPN is closely related to NF-κB. High blood sugar levels can lead to oxidative stress, increasing the activity of NF-κB, and subsequently causing the expression of NF-κB subunit P65 as an important metabolic mechanism (13). On the basis of controlling blood sugar in diabetes patients with DPN, zinc sulfate treatment can contact the inflammatory memory function of the body. Drug treatment can significantly reduce the oxidative stress state of the body of DM patients, which inhibits NF-κB, reduces the inflammatory reaction of the body, and plays a protective role on pathological histiocytes (14).

miR-146a owns an essential role in the occurrence of inflammation, tumor diseases, and immune diseases in the body, and inflammation is one of the important conditions that regulate miR-146a (15). miR-146a with dysregulated expression will indirectly affect NF-κB by targeting IRAK1 and TRAF6 proteins mechanism, which occurs a major role in regulating the occurrence of DPN.miR-146a is mainly located in the second exon of the LOC285628 gene on chromosome 5 in the human body. The gene contains a pre-miR-146aC/G variant gene encoding 2910164. The base substitutions of the G to C genotypes mentioned above are mainly located in the hairpin structure of the pre-miRRNA follower chain, which will have a serious impact on the efficiency of generating nucleus pre-miR-146 and the mature expression of miR-146a. This will lead to a decrease in free energy, further affecting the mature expression of miR-146a (16). There is a close relationship between the polymorphism of the Rs910164 gene and the expression level of miR-146a. The expression level of miR-146a is lower in GG-type patients than in CC-type genes, and the risk of DPN is significantly increased (17). This study showed that the allele G and GG genotype frequencies in the zinc sulfate treatment effective group were lower than those in the ineffective group, while the allele C and CC gene frequencies in the invalid set were higher. This indicates that the CC genotype has a significant effect in zinc sulfate drug therapy, but the treatment effect on GG genotype is not ideal.

DPN still lacks specific treatment plans and methods in clinical practice, and currently, only symptomatic treatment is carried out through blood sugar control and drug nutrition for nerve cells. However, this treatment plan can only alleviate clinical symptoms and signs, and has lower

Table 4. Comparison of TCSS scores of DPN patients with different miR-146a genotypes ($\bar{x}\pm s$, points).

Different miR-146a genotypes	n	TCSS score (points)		*t*	*P*
		before treatment	after treatment		
GG	40	9.18±1.20	6.98±1.14	8.406	0.000
GC	38	9.21±1.25	6.05±1.10	12.003	0.000
CC	38	9.25±1.22	5.28±1.16	14.915	0.000

significance in improving the condition. Zinc sulfate, as a coenzyme, exists in the mitochondrial enzyme and can be biotransformation into reduced dihydrogen sulfate (18). The application of sufficient doses of zinc sulfate can effectively eliminate free radicals and chelated metal ions in the body, enabling them to generate other antioxidants and have significant antioxidant effects. When drugs exert their effects, they can inhibit the oxidative stress state of nerves, accelerate cell metabolism, promote tissue blood flow rate, and help improve nerve conduction velocity (19). Zinc sulfate, as a powerful antioxidant with multiple functions, can prevent the glycosylation of proteins in the body. At the same time, it can also inhibit aldose reductase, thereby reducing the production of sorbitol and improving the clinical symptoms of DPN (8). This research survey shows that after treatment, the levels of MNCV and SNCV of CC genotype median nerve and common peroneal nerve are higher than those before treatment. This indicates that zinc sulfate therapy has the most significant effect on the recovery of nerve conduction velocity in CC genotype patients among the three genotypes, and zinc sulfate therapy can be used alone for treatment (20). The improvement of nerve conduction velocity in CG and GG genotype patients is not significant, indicating that miR-146a polymorphism can affect the effectiveness of drug treatment. On the basis of implementing zinc sulfate drug therapy for two non-effective genotypes, genotype factors should be considered and other drug combinations should be added to improve treatment effectiveness. After treatment, the TCSS scores of DPN patients with various genotypes were lower than before treatment. This indicates that zinc sulfate treatment can improve disease symptoms to a certain extent in all genotypes of DPN patients with T2DM, and also has a certain effect on prognosis and rehabilitation (21).

In summary, the miR-146a polymorphism can have a certain impact on the implementation of zinc sulfate treatment for DPN in T2DM patients, increasing disease susceptibility and reducing drug treatment efficacy. Among them, GG genotype T2DM patients have the highest susceptibility and the worst treatment effect; The susceptibility of CC genotype T2DM patients is lower, and the treatment effect is significant. The miR-146a polymorphism can serve as an early predictive indicator for the prognosis of DPN treated with zinc sulfate, providing a reference basis for disease prognosis evaluation. CC genotype patients can achieve significant results by implementing zinc sulfate treatment alone. For patients with GG and GC genotypes, early treatment with methylcobalamin can be combined to improve treatment effectiveness.

References

1. Barman S, Srinivasan K. Diabetes and zinc dyshomeostasis: Can zinc supplementation mitigate diabetic complications? Crit Rev Food Sci Nutr 2022; 62(4): 1046-1061. https://doi.org/10.1080/10408398.2020.1833178
2. Rafiullah M, Siddiqui K. Pharmacological Treatment of Diabetic Peripheral Neuropathy: An Update. CNS Neurol Disord Drug Targets 2022; 21(10): 884-900. https://doi.org/10.2174/1871527320666210303111939
3. Alhakamy NA, Mohamed GA, Fahmy UA, Eid BG, Al-Rabia MW, Khedr AIM, Nasrullah MZ, Ibrahim SRM, Abdel-Naim AB, Ahmed OAA, Md S. Thioctamer: a novel thioctic acid-glatiramer acetate nanoconjugate expedites wound healing in diabetic rats. Drug Deliv 2022; 29(1): 1776-1784. https://doi.org/10.1080/10717544.2022.2081382
4. Liu Y, Qin L, Wang J, Xie X, Zhang Y, Li C, Guan Z, Qian L, Chen L, Hu J, Meng S. miR-146a Maintains Immune Tolerance of Kupffer Cells and Facilitates Hepatitis B Virus Persistence in Mice. J Immunol 2022; 208(11): 2558-2572. https://doi.org/10.4049/jimmunol.2100618
5. Zhang J, Xian TZ, Wu MX, Li C, Wang W, Man F, Zhang X, Wang X, Pan Q, Guo L. Comparing the effects of twice-daily exenatide and insulin on renal function in patients with type 2 diabetes mellitus: secondary analysis of a randomized controlled trial. J Investig Med 2022; 70(7): 1529-1535. https://doi.org/10.1136/jim-2021-002237
6. González-González JG, Violante-Cumpa JR, Zambrano-Lucio M, Burciaga-Jimenez E, Castillo-Morales PL, Garcia-Campa M, Solis RC, González-Colmenero AD, Rodríguez-Gutiérrez R. HOMA-IR as a predictor of Health Outcomes in Patients with Metabolic Risk Factors: A Systematic Review and Meta-analysis. High Blood Press Cardiovasc Prev 2022; 29(6): 547-564. https://doi.org/10.1007/s40292-022-00542-5
7. Kazemi S, Afshar S, Keramat F, Saidijam M, Karami M, Hashemi SH, Alikhani MY. Assessment of Association between miR-146a Polymorphisms and Expression of miR-146a, TRAF-6, and IRAK-1 Genes in Patients with Brucellosis. Mol Biol Rep 2022; 49(3): 1995-2002. https://doi.org/10.1007/s11033-021-07014-4
8. Levinstein MR, Ventriglia EN, Gomez JL, Budinich RC, Marton J, Henriksen G, Holt DP, Dannals RF, Pomper MG, Zarate CA Jr, Bonaventura J, Michaelides M. 6-O-(2-[^{18}F]Fluoroethyl)-6-O-Desmethyl-Diprenorphine ([^{18}F]FE-DPN) Preferentially Binds to Mu Opioid Receptors In Vivo. Mol Imaging Biol 2023; 25(2): 384-390. https://doi.org/10.1007/s11307-022-01767-5
9. Mirzaei S, Saghari S, Bassiri F, Raesi R, Zarrabi A, Hushmandi K, Sethi G, Tergaonkar V. NF-κB as a regulator of cancer metastasis and therapy response: A focus on epithelial-mesenchymal transition. J Cell Physiol 2022; 237(7): 2770-2795. https://doi.org/10.1002/jcp.30759
10. Albarnaz JD, Ren H, Torres AA, Shmeleva EV, Melo CA, Bannister AJ, Brember MP, Chung BY, Smith GL. Molecular mimicry of NF-κB by vaccinia virus protein enables selective inhibition of antiviral responses. Nat Microbiol 2022; 7(1): 154-168. https://doi.org/10.1038/s41564-021-01004-9
11. Etoga MC, Amandine Well E, Pierre Choukem S, Dehayem M, Mendane Mekobe F, Boli Ongmeb A, Hadja Inna A, Claude Mbanya J, Sobngwi E. Short-Term Effects of Liraglutide versus Vildagliptin on Insulin Secretion and Sensitivity in Type 2 Diabetes: A Single Blinded Randomized Controlled Trial (LIRAVIS Study). J Diabetes Mellit 2023; 13(1): 45-57. https://doi.org/10.4236/jdm.2023.131005
12. Liu F, Ma F, Kong G, Wu K, Deng Z, Wang H. Zinc supplementation alleviates diabetic peripheral neuropathy by inhibiting oxidative stress and upregulating metallothionein in peripheral nerves of diabetic rats. Biol Trace Elem Res 2014; 158(2): 211-218. https://doi.org/10.1007/s12011-014-9923-9
13. Zhang X, Zhang Y, Zhou P, Ai J, Liu X, Zhang Q, Wang Z, Wang H, Zhang W, Zhang J, Huang Y. Down-regulated cylindromatosis enhances NF-κB activation and aggravates inflammation in HBV-ACLF patients. Emerg Microbes Infect 2022; 11(1): 1586-1601. https://doi.org/10.1080/22221751.2022.2077128
14. Haftcheshmeh SM, Abedi M, Mashayekhi K, Mousavi MJ, Navashenaq JG, Mohammadi A, Momtazi-Borojeni AA. Berberine as a natural modulator of inflammatory signaling pathways in the immune system: Focus on NF-κB, JAK/STAT, and MAPK signaling pathways. Phytother Res 2022; 36(3): 1216-1230. https://doi.org/10.1002/ptr.7407

15. Hu Y, Zhang Q, Wang JC, Wang J, Liu Y, Zhu LY, Xu JX. Resveratrol improves diabetes-induced cognitive dysfunction in part through the miR-146a-5p/TXNIP axis. Kaohsiung J Med Sci 2023; 39(4): 404-415. https://doi.org/10.1002/kjm2.12643
16. Chen X, Xie L, Jiang Y, Zhang R, Wu W. LCK, FOXC1 and hsa-miR-146a-5p as potential immune effector molecules associated with rheumatoid arthritis. Biomarkers 2023; 28(1): 130-138. https://doi.org/10.1080/1354750X.2022.2150315
17. Li Z, Liu G, Huang H. Silencing of long non-coding RNA SOX2-overlapping transcript relieves myocardial ischemia/reperfusion injury through up-regulating miRNA-146a-5p. Gen Physiol Biophys 2023; 42(2): 191-199. https://doi.org/10.4149/gpb_2022065
18. Ren L, Guo R, Fu G, Zhang J, Wang Q. The efficacy and safety of massage adjuvant therapy in the treatment of diabetic peripheral neuropathy: A protocol for systematic review and meta-analysis of randomized controlled trials. Medicine (Baltimore) 2022; 101(10): e29032. https://doi.org/10.1097/MD.0000000000029032
19. Jiménez-Del-Barrio S, Cadellans-Arróniz A, Ceballos-Laita L, Estébanez-de-Miguel E, López-de-Celis C, Bueno-Gracia E, Pérez-Bellmunt A. The effectiveness of manual therapy on pain, physical function, and nerve conduction studies in carpal tunnel syndrome patients: a systematic review and meta-analysis. Int Orthop 2022; 46(2): 301-312. https://doi.org/10.1007/s00264-021-05272-2
20. Meuronen T, Lankinen MA, Kärkkäinen O, Laakso M, Pihlajamäki J, Hanhineva K, Schwab U. FADS1 rs174550 genotype and high linoleic acid diet modify plasma PUFA phospholipids in a dietary intervention study. Eur J Nutr 2022; 61(2): 1109-1120. https://doi.org/10.1007/s00394-021-02722-w
21. Franceschi R, Mozzillo E, Di Candia F, Rosanio FM, Leonardi L, Liguori A, Micheli F, Cauvin V, Franzese A, Piona CA, Marcovecchio ML. A systematic review of the prevalence, risk factors and screening tools for autonomic and diabetic peripheral neuropathy in children, adolescents and young adults with type 1 diabetes. Acta Diabetol 2022; 59(3): 293-308. https://doi.org/10.1007/s00592-022-01850-x