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Research Article

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Clinical and Pharmacological Basis for the Use of Drugs Inhibiting of the RAAS in Patients with Diabetic Neuropathy

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Abstract

Diabetes mellitus (DM) is the most common cause of diabetic neuropathy (DN) comprises a heterogeneous group of disorders that can cause neuronal dysfunction throughout the human body. The incidence of diabetes and its complications is increasing to staggering proportions. In 2014 the WHO estimated an overall prevalence of 422 million (8, 5%). The incidence of diabetic neuropathy approaches 50% in most diabetic populations; there is no treatment, and its consequences in the form of foot ulceration and amputation.

The recent studies suggest that the renin angiotensin aldosterone system (RAAS) plays a vital role in regulating glucose metabolism and blood pressure. In the same time the metabolic abnormalities associated with diabetes lead to activation RAAS, which might promote the formation of reactive oxygen species to lead the endothelial and neuronal dysfunctions. Furthermore, TNFα is part of the response of the organism to hypertension and is originally described as one of the central mediators of inflammation trough the activation of transcription factor NFκB an important factor in the control of cell proliferation, differentiation, and apoptosis.

***Methodology & Theoretical Orientation:** The study is going on in parallel groups. The patients (enrolled on randomized principle) with DPN will be investigated. The enrolled subjects was divided into two main groups: group I with Type I and Type II DM, complicated by DPN to take Aliskiren and group II with the same pathology, proceeding with the treatment without Aliskiren but given Telmisartan, for certainty of Aliskiren efficacy. At the start of the trial and on completion of the six week period TNFα level and C-peptide will be determined.*

***Findings:** Telmisartan has less TNFα modulatory effects then Aliskiren, Namely, the symptoms of neuropathy a well as blood TNFα level and C-peptide level are not changed significantly.*

***Conclusion & Significance:** TNFα is involved in DPN pathogenesis formation and clinical manifestation. Aliskiren ameliorates symptoms in DPN patients by modulatory impact on TNFα, so we have results for clinical and pharmacological analysis of Aliskiren application in DPN. The involvements of RAAS system in developments of DNP needs further research study.*

Keywords: Aliskiren, Telmisartan, Diabetes Mellitus, Diabetic Neuropathy, Renin Angiotensin Aldosterone System, Tumor Necrosis Factor Alpha, Peroxisome Proliferator-Activated Receptor Gamma.

Introduction

Diabetes mellitus (DM) is the main problem of public health worldwide. The incidence of diabetes and its complications is increasing to staggering proportions. In 2014 the WHO estimated an overall prevalence of 422 million (8, 5%). In 2015, 1.6 million deaths were directly caused by diabetes [1]. It is expected there will be 552 million people with diabetes until 2030 [2]. DM patients have a high risk to develop microvascular complications such as retinopathy, and neuropathy [3]. The incidence of diabetic neuropathy approaches 50% in most diabetic populations; there is no treatment, and its consequences in the form of foot ulceration and amputation are financially punishing for health care providers [4].

The treatment of diabetic neuropathy (DN) still remains unresolved. There are currently no FDA-approved therapies for DN and only 3 approved therapies for painful DN. But unfortunately no treatment results in complete resolution of the underlying pathophysiological abnormalities and treatment of DN is an unmet need in clinical practice. Only strict metabolic control appears to have a beneficial effect on the prevention and delay of the onset of DN and to reduce the prevalence of established DN [5-8]. Nowadays the optimal therapy involves: blood glucose level control, anticonvulsants,

antidepressants and opioid administration, though it does not change pathogenic pattern. In addition of that patients have high need for meticulous foot hygiene, appropriate footwear, and mobility support as needed [9-11].

DN includes a heterogeneous group of disorders that can cause neuronal dysfunction throughout the human body [12]. In about one fifth of patients, painful diabetic peripheral neuropathy (DPN) predominates, and has a significant negative impact on health-related quality of life and general function [13]. Foot problems from underlying DPN are a major cause for developing ulcers, Charcot foot abnormalities, injuries, infections, and lower extremity amputation and this is a lifetime risk for patients with DM [14]. Other manifestations include small-fiber neuropathy, autonomic neuropathy, diabetic amyotrophic, radiculopathy, mononeuritis multiplex, mononeuropathy, and treatment-induced neuropathy [15].

DM has multi-component pathogenesis, varied clinical manifestations, and is characterized by diverse mechanisms of development, clinical course and complications. In Type I diabetes, the immune system attacks and affects insulin-producing beta cells in the pancreas. It is characterized by the formation of autoantibodies, progressive immune cell infiltration in Langerhans islets, followed by insulin-producing beta-cell destruction [16]. It is believed that Cytokine production may be the major factor in beta-cell death involving interleukin1 (II-1), interferon γ (INF γ) and tumor necrosis alpha (TNF-α) [17, 18] belonging to inflammatory cytokines produced by T cells during this process. It has been identified that TNF-α and RAAS play a significant role in the development of Type II diabetes through the resistance formation to insulin [19, 20]. TNF-α mainly produced in adipocytes and/or peripheral tissues induces tissue-specific inflammation through the involvement of generation of ROS and activation of various transcriptional mediated pathways. One of the results of such effects is formation of insulin resistance through serine phosphorylation that leads to the development of T2DM [21]. The data collected in the present-day scientific literature indicate the essential pathogenic role of TNFα in the development of diabetic neuropathy (DNP) [22].

Generally, two mechanisms have been suggested to be involved in the pathogenesis of DPN. The first mechanism is the activation of the RAAS in the presence of hyperglycemia with increased tissue level of Ang II. Ang II stimulates NAD (P) oxidase which enhances oxidative stress and vascular damage and leading to DPN [23]. The other mechanism is disturbance in the metabolism and vasculature of nerve tissue in the presence of excessive uptake of glucose [24].

The recent studies suggest that RAAS plays a vital role also in regulating glucose metabolism and blood pressure, electrolyte and fluid homeostasis. The clinical research results indicate that the genes of RAAS have important roles in glucose metabolism and regulation of blood pressure [2]. Consequently, RAAS is associated with DM and its complications of retinopathy, neuropathy and cardiovascular disease (CVD) [25]. In the same time the metabolic abnormalities associated with diabetic patients hyperglycemia lead to activation RAAS, which might promote the formation of reactive oxygen species to lead the endothelial dysfunctions, thrombosis, inflammation and vascular remodeling [26, 27]. Further, Ang II increases reactive oxygen species, which leads to damaging the pancreatic β-cells and may indirectly impair insulin secretion from the pancreas through vasoconstriction and reduction in islet blood flow [28]. The other component of the RAAS, aldosterone, decreases the insulin secretion from β-cells in a mechanism involves oxidative stress [29]. On other hand TNFa is part of the response of the organism to hypertension. TNFα was originally described as one of the central mediators of immunity and inflammation trough the activation of transcription factor NFκB an important factor in the control of cell proliferation, differentiation, and apoptosis through the induction of variety of genes [30]. Moreover, local accumulation of glucose and its metabolite, succinate, through activation of a G-protein coupled receptor (CPR91) triggers the cell to cell signaling that results in prorenin and renin release from juxtaglomerular cells in early diabetes [4].

In the same time the damage of peripheral nerve in diabetes could be attributed to polyol accumulation, advanced glycation end-products and oxidative stress [23]. Formation of advanced glycosylated end products (AGEs) in DM appears to play a crucial role in the pathogenesis of microvascular complications. Namely, it has been proposed that the pathophysiological cascades triggered by AGEs have a dominant, hyperglycemia-independent role in the onset of the microvascular complications of diabetes [31].

Furthermore, Onset of insulin resistance is often accompanied by obesity, in particular visceral obesity. Resistance of dysfunctional fat cells to the antilipolytic effects of insulin leads to chronic elevations in plasma free fatty acid (FFA) levels. This, in turn, induces insulin resistance in the liver and skeletal muscle, resulting in reduced glucose uptake and increased gluconeogenesis. Dysfunctional fat cells also produce excessive amounts of cytokines (e.g., tumor necrosis factor-α [TNF-α], interleukin [IL]-6, and resisting) that further induce insulin resistance, inflammation, and atherosclerosis and that secrete reduced amounts of insulin-sensitizing cytokines such as adiponectin [32].

As the recent clinical studies shows the peroxisome proliferator-activated receptor (PPAR) family of nuclear receptors and the winged-helix-fork head box class O (FOXO) family of factors are two key families of transcription factors that regulate glucose homeostasis and insulin responsiveness in the adipose and muscle tissues [33]. The three peroxisome proliferator–activated receptor (PPAR) isoforms PPARα, -γ, and -δ are nuclear receptors activated by fatty acids and fatty acid–derived eicosanoids [32, 34]. PPARγ was the first gene reproducibly associated with T2DM [35, 36]. PPARγ activation can regulate gene expression for genes involved in metabolism of glucose and lipids, insulin's sensitivity, cell growth and differentiation [37, 38]. PPARγ is also expressed in immune/inflammatory cells (monocytes and macrophages), which could contribute to its anti-inflammatory activity [39, 40]. Consequently, PPARγ has been the focus of intense research during the past decade because ligands for this receptor have emerged as potent insulin sensitizers used in the treatment of T2DM [41].

Finally it is also very important to identify that C-peptide deficiency is an important contributing factor to the characteristic functional and structural abnormalities of the peripheral nerves [42]. C-peptide binds to cell membranes, resulting in stimulation of endothelial nitric oxide synthase (eNOS) and Na+, K+-ATPase [43].

As it is discussed a number of different therapeutic approaches that target the various pathogenetic mechanisms of diabetic neuropathy have been the subject of clinical trials [44].

The many literature sources suggests that the various mechanisms associated with elevation of TNFα can be cause of the inflammatory damages leads to formation of insulin resistance, pancreatic β-cell apoptosis, as well as formation of DPN in patients with T2DM [22, 45-48]. In consequence, further optimization of the syndrome treatment by inhibition of TNF-α will become feasible.

Also it has been demonstrated that the inhibition of RAAS prevents the adverse effects of Ang II on glucose metabolism and insulin resistance [28]. Results of a meta-analysis indicated that the treatment of no diabetic individuals with RAAS inhibitors decreased the risk of T2DM [12]. Decreased production of Ang II and aldosterone has been improved insulin sensitivity in both in vivo and in vitro studies [29].

Current clinical researches confirm that Renin inhibitor Aliskiren regulates inflammation by reducing MAPK (mitogen activated protein kinase, activated by renin) and Nuclear Factor B (NF-k B) activation in kidneys [11]. It is noteworthy that Aliskiren serves as TNF-α activation modulator [49]. In addition of that the recent clinical studies indicates significant effects of Aliskiren treatment in diabetic nephropathy (DN) in rats, which could be attributed to its anti-diabetic, Reno protective, antioxidant, anti-inflammatory, and anti-apoptotic effects. Aliskiren normalized streptozotocin-induced hyperglycemia in rats, increased insulin level both in vivo and in vitro [50]. Aliskiren as a direct renin inhibitor is non-peptide molecule marked by high affinity and specificity to human renin. And leads to inhibition of renin-angiotensin system at its activation stage, respectively, angiotensin I, angiotensin II and aldosterone levels as well as hemodynamic and inflammatory effects induced by them decrease. Therefore, it can become a reasonable therapeutic choice to treat a wide range of clinical conditions like cardiovascular and cardio-renal diseases, diabetes and peripheral artery diseases [51].

In addition of that the research study conducted on the rats indicates that, Telmisartan (AT1R blocker) has a potential neuro-protective effect on peripheral DN; this is mediated through its anti-inflammatory effects and its dual properties as an angiotensin receptor blocker, and a partial peroxisome proliferator activator receptor-g ligand [52, 53].

It can be assumed that administration of RAAS inhibitors will significantly improve patients' condition by modulating TNFα activation and correspondingly, reduce severity of neuropathy, extent and number of complications. Quality of life will improve and consequently mortality rate and health care expenditures will decrease

Research goals and objectives

Research goal: Assessment of Aliskiren and Telmisartan efficacy in clinical management of diabetes mellitus, complicated with peripheral neuropathy

We hypothesized that administration of Aliskiren and Telmisartan in patients with DPN during 6 weeks would ameliorate the symptoms of DNP trough blocking of RAAS and consequently inhibition of TNF-α level, as well as by activation PPARG which would contribute to the prevention of DPN induced complication in patients with Diabetes Mellitus.

Methods

Participants

Total 30 patients (over 18 years of age) with Diabetes Mellitus complicated by DPN who fulfilled inclusion criteria were randomized enrolled in this study and were informed of study requirements from internal medicine department #2 of Tbilisi State Medical University Hospital. The following exclusion criteria were applied to select the participants; (1) Subjects on ACE inhibitors and other RAAS inhibitors; (2) Subjects with the history of myocardial infarction, cardiovascular surgical intervention, acute coronary syndrome, atrial fibrillation, dysrhythmia, severe cardiac ischemia; (3) Subjects with renal failure (creatinine level over 1.5mg.dL); (4) Subjects with hyperkalemia over 0.5 mmol/L. We used trial Termination Criteria for Patients, in Accordance with GCP (Good Clinical Practice) Standards.

Methods applied

Each lab test was conducted at 9.00 am. Blood HbA1c was measured using "Ge Tein BioMedical inc" Immune chromatography diagnostic test kits and medical devices; but for assessment of fasting C-peptide and Plasma TNF-α level we have used immunoassay method using AccuBind ELIZA kits ("Monobind inc") and "Immun Diagnostik" TNF-α ELISA kits respectively. The results have been analyzed by the reader-"Urit Medical 600".

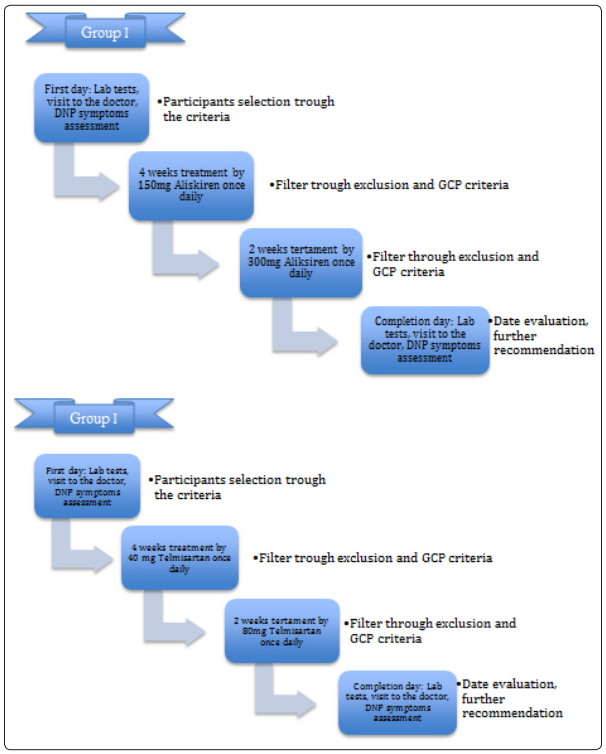
We have evaluated severity of DPN in patients by revised neuropathy disability score (NDS) [54]. which includes assessment of the following parameters: (a) Vibration perception threshold (128Hz); (b) Temperature perception; (c) Pin prink testing; (d) Achilles tendon reflex; The maximum score for the modified NDS is 10 (0 if normal; 1 if abnormal for a-c and if the reflex present (score=0), present with reinforcement (score=1) or absent (score-=2) for d) indicating a complete loss of all sensory modalities and absent reflexes. A score of six or more has been found to indicate an increased risk of foot ulceration. Also we used additionally Monofilament sensory testing and added max 2 score (0 if normal; 1 if abnormal for each foot).

Study design

This study was randomized, open-label, within-participants of clinical trial and was conducted according to the study protocol involving human subjects was approved by ethnic committee of Tbilisi State medical University. At the beginning of the trial the study protocols and benefits of study were explained to each participant. The written informed agreement was signed by all of participants. On the first day, before medical consultation patients passed through lab investigation to perform blood HbAc, serum TNFα and fasting C-peptide tests. During the medical consultation the subjects with DPN were detailed examined through: (a) the general inspection of the feet and patient's foot wear; (b) vascular asses of the feet, and assessment of the heart rate and blood pressure (c) neurological assessment by the above mentioned methods (see 2.2).

The enrolled subjects was divided into two main groups: group I composed by 15 patients with Type I and Type II diabetes mellitus, complicated by DPN to take Aliskiren during first 4 weeks in dose 150mg and in dose 300mg daily following two weeks, and group II formed by 15 patients with the same pathology, proceeding with the treatment without Aliskiren but given Telmisartan 40mg and 80 mg respectively for certainty of Aliskiren efficacy.

At the end of trail all of patients did serum TNF-α and fasting C-peptide tests as well as are passed through clinical evaluation to detect severity of DPN using revised neuropathy disability score (NDS). During the clinical study period patients are filtered through GCP criteria monitor by study implementation team (Figure 1.1).



Results

Group I

Of 15 subjects (mean age 63.3), suffered from DNP, which consisted mild pain in 67% (n=10), severe in 13% (n=2) and painless in 20% (n=3) were evaluated. The majority of patients 64% (n=9) were diagnosed less than 10 years ago. Of the 5 patients, 36% have had diagnosed for 15 years or more. Patients were Caucasians and over half were male (60%); all of subjects in this study had co-morbidity: hypertension (no hyperlipidemia) (26%), hyperlipidemia (no hypertension) (1%) and obesity (73%). All of patients were evaluated for HbA1c at the beginning. The mean of HbA1c was 6.8%. Of the 15 patients 46% (n=7) have HbA1c of 7.0% or less, and 53% (n=8) of the patients had HbA1c greater than 9.0% (table 1.1). Because one of patient violated terms and conditions laid down in the protocol the termination criteria filter excludes him from study. 14 patients completed pilot phase of clinical trials.

Table 1.1: Baseline characteristics of study participants (Group I_n=15)

Characteristics	Participants, n (%)
Men	9 (60)
Women	6 (40)
Mean age, years	63.3
Mean body weight (kg)	78.6
Race/ethnicity	Caucasians
Smoking status	
Smokers	5 (33)
Nonsmokers	10 (67)
T2DM alone (no hypertension or hyperlipidemia)	0
T2DM + hypertension (no hyperlipidemia)	4 (26)
T2DM + hyperlipidemia (no hypertension)	1 (6)
Obesity	11(73)
HbA1c start level	
Mean	6.8
HbA1c 7% or lesser	7 (46)•
HbA1c 9% or greater	8 (53)
Comorbidity	
None	0
Depression	4 (26)
DNP	15 (100)
Total NDS ≥6	12(20)
Total NDS<6	3(20)
Total NDS=8	2(13)
Painless DNP	3(20)
Mild pain DNP	10
Severe pain DNP	2
Retinopathy	2 (20)
Chronic kidney disease	1 (6)

• Percentages may not total to 100 because of rounding

The plasma TNF-α level was high (μ=25.1 pg/ml) in patients with NDS ≥6 (n=12). After 6 weeks Aliskiren treatment the serum TNF-α level was changed 25.1±7.3pg/ml vs 17.4±2.9pg/ml as shown in (Figure 2.1). The baseline of serum TNF-α μ=25.1 pg/ml significantly changed in study participants after Aliskiren treatment μ=17 pg/ml. The serum TNF-α levels of patients with DNP before and after Aliskiren application was changed by ≤45% (n=12) and by >45% (n-2); p=0.0122 (<0.05);

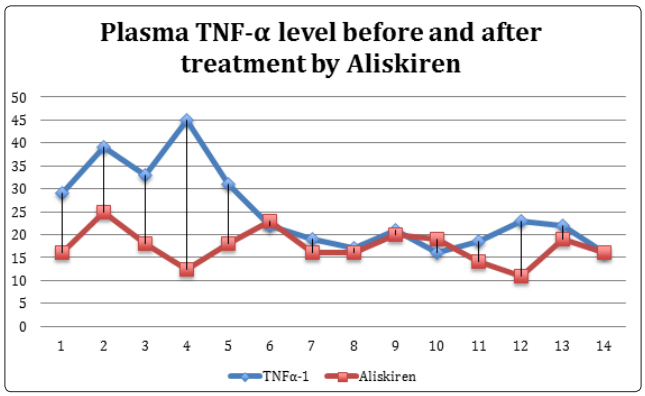


Figure 2.1: Plasma TNF- α level profiles of Aliskiren treatment during the study period in participants with DNP (n=14); the blue line is plasma TNF- α level (μ=25, 1 pg/ml) before treatment, red line indicates how Aliskiren reduces plasma TNF- α level (μ=17.4 pg/ml) after 6 weeks treatment.

In the same time the fasting C-peptide level was changed 2.3±0.7 ng/ml vs. 2.9±0.8 ng/ml after 6 weeks Aliskiren treatment as indicated in (Figure 3.1). In the most patients of group the fasting insulin C-peptide levels before and after Aliskiren application was modified by ≤ 45%(n=11) and in some patients by >45% (n-3); p=0.039 (<0.05);

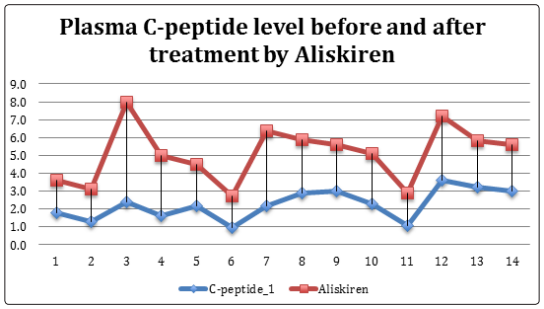


Figure 3.1: Fasting insulin C-peptide level profiles of Aliskiren treatment during the study period in participants with DNP (n=14); the blue line is fasting C-peptide (μ=2.3ng/ml) before treatment, red line indicates how Aliskiren regulates plasma C-peptide level (μ=2.9ng/ml) in each patient (n=14) after 6 weeks treatment

Consequently, DNP symptoms are improved in participants also. At the beginning of study in 20% (n=3) we have detected the score <6 and 73% (n=11) patients we have found the score ≥6 (table 1). After Aliskiren treatment DNP symptoms improved in 85% (12 from 14) and the NDS reduced <6.

Group II

Of 15 subjects (mean age 63.6 and mean body weight 87.7), suffered from DPN, which consisted mild pain in 73% (n=11), severe in 6% (n=1) and painless in 20% (n=3) were evaluated. The majority of patients 73% (n=11) were diagnosed more than 10 years ago. Of the 5 patients, 36% have had diagnosed for 4 years or less. Patients were Caucasians and over half were male (60%); all of subjects in this study had co-morbidity: hypertension (no hyperlipidemia) (53%), hyperlipidemia with hypertension (67%) and obesity (53%). All of patients were evaluated for HbA1c at the beginning. The mean of HbA1c was 7.69%. Of the 15 patients 33% (n=5) have HbA1c of 7.0% or less, and 20% (n=3) of the patients had HbA1c greater than 9.0% (table 1.2). Because one of patient violated terms and conditions laid down in the protocol the termination criteria filter excludes him from study. 14 patients completed pilot phase of clinical trials.

Table 1.2: Baseline characteristics of study participants (and Group II_n=15)

Characteristics	Participants, n (%)
Men	9 (60)
Women	6 (40)
Mean age, years	63.6
Mean body weight (kg)	87.7
Race/ethnicity	Caucasians
Smoking status	
Smokers	5 (33)
Nonsmokers	10 (67)
T2DM insulin dependent	1 (6)
T2DM alone (no hypertension or hyperlipidemia)	1 (6)
T2DM + hypertension (no hyperlipidemia)	8 (53)
T2DM + hyperlipidemia +hypertension	10 (67)
Obesity	8(53)
HbA1c start level	
Mean	7.69
HbA1c 7% or lesser	5 (33)•
HbA1c 9% or greater	3 (20)
Comorbidity	
None	0
Depression	3 (20)
DNP	15 (100)
Total NDS ≥6	8(53)
Total NDS<6	7(47)
Total NDS=8	5(33)
Painless DNP	3(20)
Mild pain DNP	11 (67)
Severe pain DNP	1 (13)
Retinopathy	10 (67)
Chronic kidney disease	2 (13)

• Percentages may not total to 100 because of rounding

The plasma TNF-α level was high (μ=34.8 pg/ml) in patients with NDS =8 (n=5). After 6 weeks Telmisartan treatment the serum TNF-α level was changed 24.5±9.9pg/ml vs 16.8±3.0pg/ml as shown in (Figure 2.2). The baseline of serum TNF-α μ=24.5 pg/ml significantly changed in study participants after Telmisartan treatment μ=16.8 pg/ml. The serum TNF-α levels of patients with DNP before and after Telmisartan application was changed by ≤40% (n=11) and by >40% (n-3); P –value is <0.00001 (p<0.05); In the same time the fasting C-peptide level was changed 2.7±1.5 ng/ml vs. 2.5±0.85 ng/ml after 6 weeks Telmisartan treatment as indicated in (Figure 3.2). In the most patients of group the fasting insulin C-peptide levels before and after Telmisartan application was modified by ≤ 20%(n=10) and in some patients by >20% (n-4); P-value is 0.103, therefore the result is not significant at p<0.05;

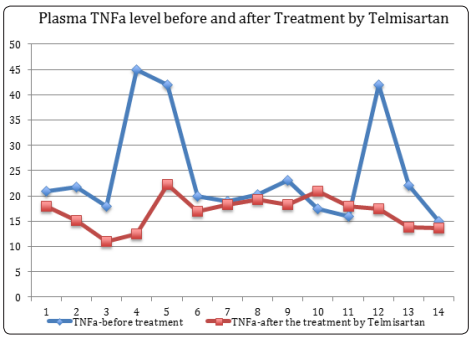


Figure 2.2: Plasma TNF- α level profiles of Telmisatan treatment during the study period in participants with DNP (n=14); the blue line is plasma TNF- α level (μ=24, 5 pg/ml) before treatment, red line indicates how Telmisartan reduces plasma TNF- α level (μ=16.8 pg/ml) after 6 weeks treatment.

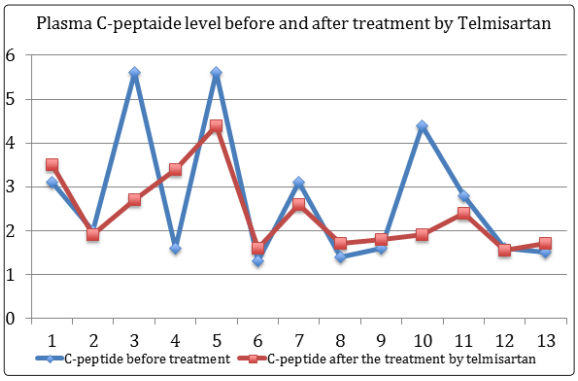


Figure 3.2: Fasting insulin C-peptide level profiles of Telmisartan treatment during the study period in participants with DNP (n=14); the blue line is fasting C-peptide (μ=2.7ng/ml) before treatment, red line indicates how Telmisartan regulates plasma C-peptide level (μ=2.5ng/ml) in each patient (n=14) after 6 weeks treatment

Consequently, DNP symptoms are improved moderately in participants. At the beginning of study in 47% (n=7) we have detected the score <6 and 53% (n=8) patients we have found the score ≥6 (table 1). After Telmisartan treatment DNP symptoms improved in 54% (4 from 8) and the NDS reduced fewer than 6.

Discussion

The main and original finding of this study was to show effects of RAAS inhibitors in DPN patients by inhibition of TNF-α, inflammatory cytokine involved in the pathogenesis of neuronal dysfunction and T2DM formation [19, 46]. We demonstrated that the administration of Aliskiren changes the serum TNF-α and C-peptide levels as well as ameliorate DNP symptoms. The level of serum TNF-α is reduced in patients with high TNFα after 6 weeks Aliskiren treatment. The TNFα inhibitory role of Asliskiren has been showed also in the recent study identified attenuated effect of Aliskiren in chronic constriction injury induced neuropathic pain and elevated TNF-α level in rats [47]. Aliskiren reduces inflammatory cytokine TNF-α activity by reducing of MPAK and NF-kB activation in the neurons as it is found in recent research [45, 55]. In addition of that Aliskiren inhibits also TNF-α activated renin, which leads MAPK induced tissues damages [56].

In the same time after 6 weeks Aliskiren treatment, the level of fasting C-peptide normalized in evaluated patients. As last evidences explain TNF-α is the main pro-inflammatory cytokine critically involved in the development of insulin resistance and pathogenesis of T2DM [19, 47]. We can propose that Aliskiren, by inhibiting of serum TNF-α level improves pancreatic β-cells function, in addition of that by inhibiting of serum TNF-α, it improves insulin receptors sensitivity and by this way reduce fasting C-peptide high level.
In addition of that clinical research studies results show that TNF-α produced by the activated macrophages and monocytes plays an important role in pathogenesis of DPN trough the demyelination of nerve fibers, disorganization of lamellar and axonal structures and decreased expression of myelin basic protein (MBP) in the nerve tissues [46]. Also, TNF-α, produced by adipocytes and/or peripheral tissues maybe cause of DNP trough generation ROS [48]. By our study we correspond to the already done clinical studies that the TNF-α high serum level and abnormal fasting C-peptide level are involved in DNP formation [46, 48, 57]. Aliskiren by modulation of the both parameters level improves the symptoms of DNP.

Moreover, recent studies results that the RAAS effects on insulin secretion as well as insulin resistance trough the formation of reactive oxygen species and leads T2DM and its cardiovascular and renal complications [58-60]. Therefore still now clinical studies tray to find association of RAAS genes with DM and its complication of retinopathy, neuropathy and cardiovascular disease [2]. In this regard we have results indicated that the inhibition of RAAS by Aliskiren trough renin inhibition plays significant role in improvement of symptoms of DPN and ameliorates insulin C-peptide level.

On the other hand this study demonstrates that the administration of Telmisartan change the serum TNF-α and moderately ameliorate DPN symptoms. The level of serum TNF-α is reduced in patients with high TNFα after 6 weeks Telmisartan treatment. Recent study indicates that Telmisartan reduces inflammatory cytokines level by inhibiting of Ag II effect in [61]. In the same time Telmisartan by activation of PPAR-γ inhibits NF-kB signaling pathway, therefore the inflammatory cytokines such as TNFa formations as well as ant apoptotic mediators' gene expression are decreased; moreover the oxidative stress elements such as reactive oxygen species and nitric oxide levels are reduced also. In contrast, antioxidants level is increased. Net effect of mentioned changes leads to amelioration of neuroinflammation [62]. Furthermore, by activating of peroxisome proliferator activator receptor-g it regulates lipid and glucose metabolism [52, 53, 63]. As a result of this action Telmisartan inhibits oxidative stress and reduces free radicals [63-65]. PPARG ligands can reduce the expression of pro inflammatory genes, decrease TNFa production and increase adiponectine expression [33, 37-41]

As we proposed that Telmisartan, by inhibiting of serum TNF-α level should improve pancreatic β-cells function, as well as insulin receptors sensitivity and by this way should reduce fasting C-peptide high level. But after 6 weeks Telmisartan treatment, the level of fasting C-peptide not normalized properly in evaluated patients. We propose that the reason of this results maybe the following, as recent study suggest, RAAS blockers prevent insulin resistance in some, but not all T2DM patients indicating inter-individual variability [2]. In addition of that formation of C-peptide takes place in the endoplasmic reticulum of pancreatic β-cells. The endoplasmic reticulum stress, which can be expressed, by the TNFα, sRAGE, IL-1- β and IFN-γ is one of the causes of C-peptide deficiency. As it is mentioned Telmisaetan regulates angiotensin II induced TNFa formation, therefore the other ligand can leads to ER stress and C-peptide formation destruction [66].

Finally, the recent clinical study indicates significant effects of Aliskiren to compare with Telmisartan in DM with complication recognized to its anti-diabetic, Reno protective, antioxidant, anti-inflammatory, and anti-apoptotic effects [50]. In our study there was positive correlation between DPN symptoms and TNF-α level. The study results prove Aliskiren above mentioned effects linked with reducing plasma TNF-α, and improving insulin C-peptide level in T2DM patients. All of this changes leads to the improvement of DPN symptoms in patients treated by Aliskiren. In the same time correlation between NDS score, HBA1c and co-morbidity of patients were not statistically significant, indicating that above mentioned factors were not proven to increase DNP.

It can be assumed that Aliskiren administration will significantly improve patients' condition by modulating TNF-activation and correspondingly, reduce severity of neuropathy, extent and number of complications. Quality of life will improve and consequently mortality rate and health care expenditures will decrease.

In the same time our clinical trials results indicate moderate effect of Telmisartan in DM trough the inhibition of AT1R and activation PPARγ as it is mentioned in the current research studies [67]. So, Telmisartan reduces plasma TNF-α, in DM patients but can't change visibly fasting C-peptide level which may be explained as one of the result of ER stress induced by different ligands together with TNFa.

Finally it should be added that it is still controversial, the dual role of RAAS in different states of pain. Consequently RAAS inhibitors modulate pain by inhibiting the inflammatory cytokines, such as TNF-α. On the contrary clinical studies have shown pain-inducing action of RAAS inhibitors [68].

Conclusion

Our results may demonstrate of TNF-α and RAAS implication for organ-specific complication pathogenesis of diabetes mellitus type 2 as well as in DPN.

Findings

Aliskiren ameliorates conditions of T2DM patients with DNP better than Telmisartan. Namely, under Aliskiren treatment the symptoms of DPN are reduced, the blood TNF-α level is reduced and C-peptide level is ameliorated while Telmisartan has moderate TNFα modulatory effects but do not change C-peptide level properly; consequently Telmisartan treatment course relatively improves the symptoms of neuropathy in DM patients.

Conclusion & Significance

Our results confirm hypothesis that TNFα and RAAS may play a substantial role in the development and progression of type 2 diabetes mellitus as well as in pathogenesis of diabetic neuropathy. Aliskiren has visible modulatory impact on TNF-α to compare with Telmisartan, so we have results for clinical and pharmacological analysis of Aliskiren application in diabetic neuropathy.

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Findings

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Conflict of interest statement

The authors, their immediate families, and any research foundations with which they are affiliated have not received any financial payments or other benefits from any commercial entity related to the subject of this article.

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