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Review

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How to diagnose neuropathy in diabetes mellitus?

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ABSTRACT

Diabetic neuropathy is a common problem and can present various clinical presentations such as cranial neuropathy, radiculopathy, plexopathy, mononeuropathy, polyneuropathy and autonomic neuropathy. Clinical evaluation and use of scoring systems for the evaluation of diabetic polyneuropathy is an important step to get correct diagnosis. New informations about the diagnosis of diabetic neuropathy continue to emerge, which will lead to correct diagnosis and treatment as well. Diabetic length-dependent sensorimotor polyneuropathy (DSPN) and carpal tunnel syndrome are the most common seen problems. While acute and painful situations with motor weakness are mostly transient, sensory fibres are predominantly involved in chronic ones. Nerve conduction studies are needed to confirm diagnosis of any type of diabetic neuropathy, but they are normal in cases with small fiber involvement. The early diagnosis is crucial because it is well-known that subclinical diabetic neuropathy may be reversed or significantly improved with diabetes control. Because neuropathy at the stage in which only small fibers are affected can be reversed, it is important to diagnose DSPN in these stages. Skin biopsy taken from the dermatomal area of sural nerve, laser-doppler-imager flare technique, corneal confocal microscopy are used to assess small fibre dysfunction. The aim of the present review was to evaluate evidence-based diagnosis for any type of neuropathy seen in the patients with diabetes mellitus.

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Keywords: length-dependent sensorimotor polyneuropathy, small fiber, nerve conduction studies, neuropathic pain, scoring systems, diabetes mellitus

Introduction

Neuropathy developing secondary to diabetes is not only a late complication but also can develop at any time during the course of the disease. It should be evaluated carefully because neuropathy symptoms are associated with poor quality of life [1]. The neuropathies developing in patients with diabetes are heterogeneous and may be separated into generalized and focal/multifocal varieties [2-4]. Onset of symptoms is also variable and may be acute, subacute, or chronic in forms. Symmetric or generalized neuropathies usually present as chronic neuropathies and asymmetric ones present as acute forms with more prominent pain [2-4]. Diabetic neuropathy diagnosis needs exclusion of other causes for peripheral neuropathy and and it is also increasingly recognized in patients with prediabetes who are at high risk of

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developing diabetes mellitus (DM). As it was seen Figure.1, there are different types of neuropathy seen in diabetes. The most commonly seen and typical form is a chronic, symmetrical, length-dependent sensorimotor polyneuropathy and is thought to be the most common variety [5]. The second most commonly problem seen in the patients with diabetes is carpal tunnel syndrome [6]. Distal symmetric polyneuropathy (DSPN) may be associated with retinopathy and nephropathy for which total hyperglycemic exposure is perhaps the most important risk covariate [7, 8]. Autonomic dysfunction and neuropathic pain may develop over time as well [7]. Chronic inflammatory demyelinating polyradiculopathy (CIDP) is another problem which may be seen in diabetic patients. It is well known that similar patterns of DSPN may occur in patients without diabetes, but with other toxic or metabolic causes [7]. Although many neuropathic problems were reported in the literature, there is not any diagnostic algorithm. The presence of atypical polyneuropathies, which are different from DSPN in several important features, is another problem to be evaluated. In this report, we will test to review the old advances and present some practical clues about the diagnosis of neuropathies in patients with diabetes mellitus.

Prevalence of any Case of Neuropathy in Diabetes

Both type I and type II diabetes are the most usual cases of neuropathy worldwide. The prevalence of neuropathy changes from 50% to 90%, depending on the criteria and methods used [5, 9-11]. Almost one and half of the diabetics had classical symmetric polyneuropathy, 25% had carpal tunnel syndrome,

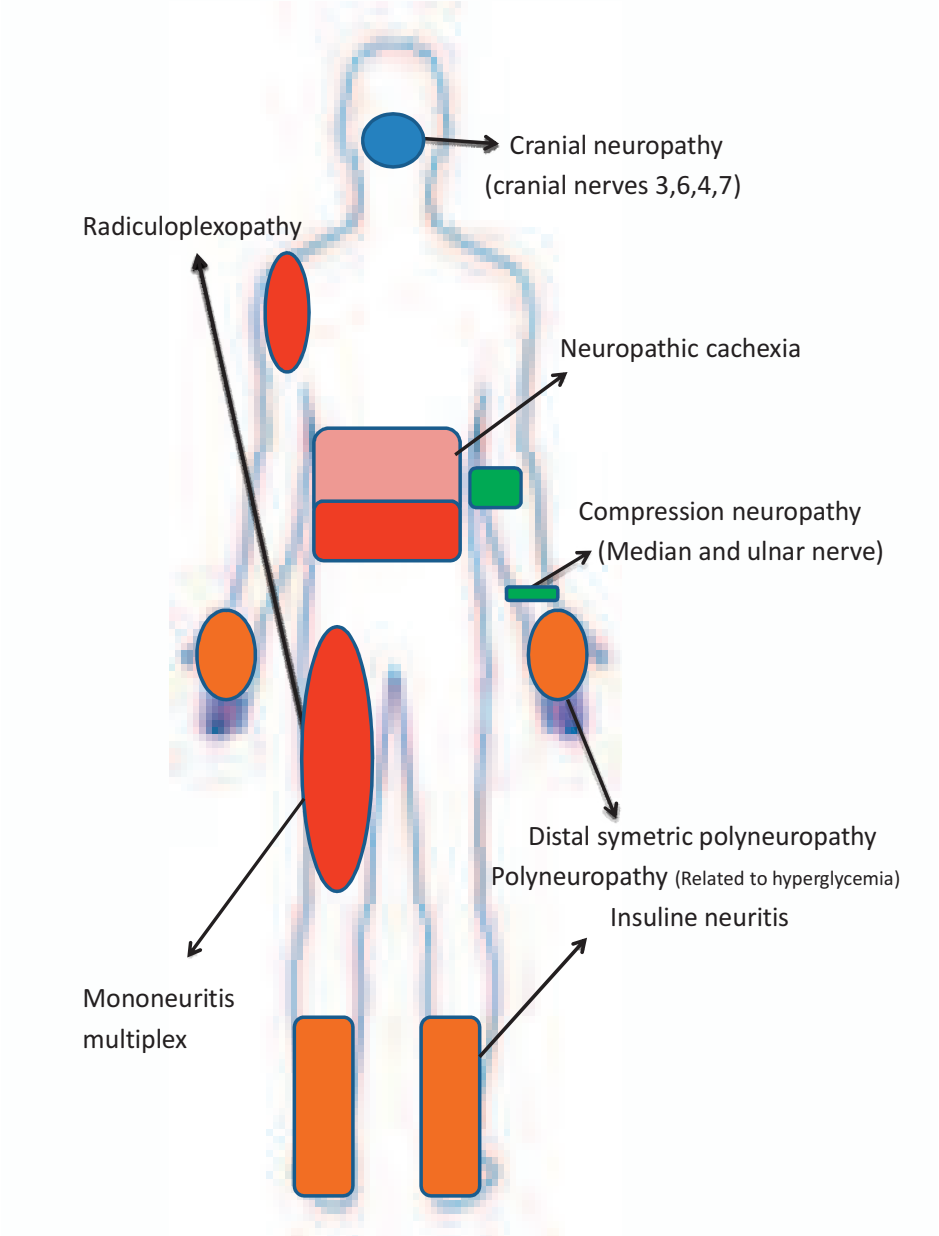

Figure 1. Types of neuropathy seen in diabetes.

about 5% had autonomic neuropathy and 1% had asymmetric proximal neuropathy [6]. The occurrence of any type of neuropathy correlates with the duration of diabetes, poor glycemic control and with the presence of retinopathy and nephropathy [5]. In patients with neuropathy associated with impaired glucose tolerance, controlled diet and exercise may lead to re-innervation [12]. Findings from subclinical neuropathy studies suggest that intensive control of glycemia may result in improved nerve conduction [13]. Neuropathic pain is a very popular and an important problem causing discomfort in patients with diabetes. Neuropathic pain is a pain arising as a direct consequence of a lesion or disease affecting the somatosensory system [14]. Bilateral thalamus, spinal cord, left parietal lobe and subcortical located deep nuclei are involved in pain generation in diabetes patients [15]. The etiology of neuropathic pain is broad, and diabetes is a significant and widely seen cause [4, 14, 15]. Neuropathic pain is also one of the

Table1. The diagnostic approaches in the patients with suspicion of diabetic neuropathy.

DIAGNOSTIC PROCEDURES	WHEN THE PROCEDURE SHOULD BE USED
Neurological Examination	1- Sensory examination: response to light touch, temperature, painful stimulus, vibration and proprioception. 2- Motor examination: testing muscle tone and strength, reflexes and coordination. 3- Autonomic findings: examing changes in colour, temperature, sweating and swelling.
Diabetic neuropathy examination (DNE) score	1- In any type of diabetic polyneuropathy
Diabetic symptom score (DNS)	1- In distal symetric polyneuropathy (DSPN)
Nerve conduction studies	4- In atypical cases with superimposed nerve entrapments or inflammatory demyelinating neuropathy such as CIDP 5- In neuropathy cases with asymmetrical symptoms 6- In cases with involvement of the upper limbs 7- In any period of DSPN (To confirm diagnosis)
Screening laboratory tests	1- Serum glucose, HbA1C ve glucose tolerance test 2- For differential diagnosis in DSPN cases Blood sugar, blood count, ESR, anti-HIV Ab, anti-Lyme Ab, Liver and renal function tests, serum vitamin B_{12}, serum protein elctrophoresis, vasculitis profile, paraneoplastic markers, thyroid function tests
Two-hour glucose tolerance testing	1- In patients with an otherwise idiopathic neuropathy 2- In patients with chronic idiopathic axonal polyneuropathy
Vibration sensation (Halux of great toe)	1- In early diagnosis of neuropathy 2- In large-fiber involved cases in diabetes 3- In all cases of other types of polyneuropathy
The Semmes-Weinstein 1-10 gram monofilament examination (SWME)	1- In early diagnosis of neuropathy 2- In large-fiber involved cases in diabetes
Genetic testing	1- In patients only with a history of hereditary neuropathy and cryptogenic polyneuropathy who exhibit a hereditary neuropathy phenotype
Neuroimaging (MRI)	1- In occulomotor nerve involved cases with pupillary involvement or absence of pain 2- In radiculopathy or plexopathy cases
Skin biopsy from the dermatomal area of sural nerve	1- In early diagnosis of DSPN to assess small-fiber function 2- In evaluation of NP development risk
Laser-Doppler-imager flare technique	1- In early and reversible stage of DSPN to assess small-fiber function (in whom results of conventional quantitative sensory tests and skin biopsy results are normal)
Neuropathic pain assessment	1- In any polyneuropathy patients to identify and quantify neuropathy
Corneal confocal microscopy	1- In early diagnosis of DSPN to assess small-fiber function 2- In evaluation of severety of neuropathy and NP development risk

Table 2. Toronto clinical neuropathy scoring system for the evaluation of diabetic polyneuropathy.

Symptom scores	Reflex scores	Sensory test scores
Foot	Knee reflexes	Pinprick
Pain	Ankle reflexes	Temperature
Numbness		Light touch
Tingling		Vibration
Weakness		Position
Ataxia		
Upper-limb symptoms		

Recording of scores (Symptom scores: present = 1; absent = 0. Reflex scores: absent = 2; reduced = 1, normal = 0. Sensory test score: abnormal = 1. normal = 0).

most common presentations not only in diabetes, but also in impaired glucose tolerance and impaired fasting glucose [4, 12, 14]. While the relationship between diabetes and neuropathic pain is well documented, the occurrence rate of neuropathic pain is still underdiagnosed and difficult to quantify [11, 15]. Neuropathic pain occurs in 7.5% to 35% of patients with DM [11, 15].

Neurological Examination and Diagnostic Procedures

Any toxic, metabolic or systemic inflammatory diseases resulting in distal type axonal neuropathies, small fiber neuropathies, demyelinating neuropathy especially CIDP, multifocal neuropathies and radiculopathies or plexopathies may mimic neuropathy seen in diabetes. Neurologic examination focuses on the recognition of clinical signs of neuropathy. Patients are asked to report whether they experienced gait problems (difficulty in walking or climbing, ataxia), thermal allodynia (e.g. When they have a bath) and/or pain in the feet. For evaluation of diabetic neuropathy, neurological examination should include cranial nerve examination, assessment of muscle force, sensations of pinprick, touch, temperature, joint position and vibration. It is also tried to identify the presence and distribution (e.g. length or non-length dependent) of any type of sensory loss and pain. At the second step, symptoms and signs of dysautonomy are recorded. Dysautonomic symptoms (e.g. pupil abnormalities, impotence, impaired bladder function, constipation or diarrhea, early satiety and gastric fullness, abnormal sweating, flushing, skin discoloration, xerostomia and xerophthalmia, orthostatic hypotension) are recorded. The examination clicks, and diagnostic processes are summarized below and some diagnostic clues are presented on Table 1. They are also detailed in the following section named as “classification and diagnostic work-up in diabetic neuropathies”.

In the algorithm, a patient with neuropathy should be assessed in order mentioned below.

1. Evaluation of neuropathic symptoms and signs:

The patients are assessed by the Toronto Clinical Scoring System for Diabetic Polyneuropathy, a validated instrument reflecting the presence and severity of DSPN as measured by sural nerve morphology and electrophysiology [16]. Sensory testing was performed on the first toe. Total scores range from normal = 0 to a maximum of 19. Mild DSPN was defined from a score of 5 onwards, and severe neuropathy as a score of 10 (see Table 2). Diabetic neuropathy examination (DNE) score, which is a modification of the Neuropathy Disability Score of Dyck is another scoring and identification way of neuropathy in which neurological examination was carried out and the neurological signs were recorded [17]. The DNE consists of eight items (Extension of the knee and dorsiflexion of the foot, Sensitivity to pinpricks at index finger and great toe, Ankle reflex, Sensitivity to touch, Vibration perception and Sensitivity to joint position). More than three abnormal results in these exams indicate presence of polyneuropathy. A third way of evaluation DSPN is to record diabetic symptom score (DNS). The DNS score has the following items: (1) unsteadiness in walking, (2) pain, burning or aching inthe legs or feet, (3) prickling sensations in the legs or feet, and (4) numbness in legs or feet. Presence is scored 1, absence 0, maximum score 4 points. The DNS is valid in cases of diabetic polyneuropathy, and is fast and easy to perform in clinical practice [18].

2. Examing muscle power and deep tendon

reflexes:

Motor and reflex exams are more important in the evaluation of other neuropathies such as radiculo/plexopathies developed secondary to diabetes. Although ankle reflex is a powerful screening tool, a combination of ankle reflex and vibration sense has superior sensitivity and specificity compared with either of them done alone for the detection of DSPN in clinical settings [19]. Muscle strength was graded using the Medical Research Council (MRC) score. Positive Romberg's sign and gait abnormalities were recorded. Ankle reflexes are obtained at both ankles. The examiner gently dorsiflex the foot and strike the achilles tendon briskly with the reflex hammer. Deep tendon reflexes were graded as normal, decreased (with reinforcement) or absent. It may be normal to have reduced or absent ankle reflexes in some elderly people, although the frequency and significance of this is disputed [20, 21]. It should be also remembered that the isolated loss of a reflex can point to a radiculopathy affecting that segment e.g. loss of ankle jerk if there is an S1 disc prolapse.

3. Quantitative sensory testing:

It is an important title meaning an objective index of neurologic functional status in the assessment of neuropathy in diabetes. The normal ranges of these tests vary systematically according to the age of the patients.These tests require the patient to be alert and cooperative for reliable results because quantitative sensory testing is a psychophysical test and lacks the objectivity of nerve conduction studies. Another problem is the weak correlation between these tests indicating the need to apply more than one test in screening for diabetic neuropathy [22].

a) Touch and pain sensation: Thouch sensation examination is performed by using cotton gauze (to test light touch and dynamic mechanic allodynia). Disposable safety needle (to test hypoalgesia, pinprick hyperalgesia) is applied for evaluation of pain sensation. Sensory loss and positive sensory signals (evoked and spontaneous pain, paresthesias) are recorded for both types of sensory modalities. Superficial pain sensation may be conducted using a sterile Neurotip (Owen Mumford, Oxford, U.K.) and it is applied at the eponychium of the first toe and it is repeated in an arrhythmic manner to the three sites described for the SWME at next paragraph and one side for the Neurotip. The superficial pain threshold is defined as the total number of times the application of the pain sensation is not perceived, with scores varying from 0 to 8 [23, 24].

b) The Semmes-Weinstein monofilament examination (SWME): It is a noninvasive, lowcost, rapid (takes less time, <5 minutes), and practical test often used in clinical testing of pressure sense [25, 26]. The 5.07/10 g Semmes Weinstein monofilament assesses the integrity of Merkel touch domes and Meissner's corpuscles and their associated large diameter fibers [27]. The variability in the methodology of SWME (weight of the filament, the number of sites, location of sites, and the diagnostic threshold) limits the effectiveness of the test as a diagnostic tool. In general, nine plantar sites (distal great toe, third toe, and fifth toe; first, third, and fifth metatarsal heads; medial foot, lateral foot, and heel) and one dorsal site were examined. The monofilaments are applied to the test site perpendicularly until they bend for about one second. Patients are instructed to say "yes" each time they sense the monofilament on their foot. If patients fail to sense the monofilament after it twists, the test site is thought to be insensate [28, 26]. It was repeated four times on both feet in an arrhythmic manner. The SWME threshold was defined as the total number of times the application of 10-g monofilament was not perceived by the patient, and it varies according to the number of placed. Patients unable to detect one or more sites should be classified as at risk in order to maximize sensitivity [26]. The quantitative analysis of the 16 studies with sufficient data revealed that SWME is both fairly sensitive and highly specific when compared with the gold standard of NCS. While the use of 5.07/10 g SWME is widespread and generally accepted, data show biothesiometry and the use of finer monofilaments (1 g) may improve detection rates of abnormality [29-31].

c) Vibration sense: Examing vibration sensation reflects large fiber involvement and it is performed by using 128 Hz Rydel-Seiffer tuning fork (vibration at the first metatarsal joints, ankles, knees, first metacarpal joints, elbows). For the best result, a 128-Hz tuning fork is applied to the halux bilaterally situated at the lateral side of the first toe [32]. The on-off method or the timed method is used in testing of vibration. It is recorded whatever the patient reports about the perception of both the start of the vibration sensation and the cessation of vibration. The testing is conducted twice on each toe, and correct responses are recorded [23, 33, 34]. The vibration can be felt for 10 to 15 seconds. Patients are asked initially, and after 5 seconds, whether they perceive vibration. Patients who felt vibration both initially and after 5 seconds are

scored as normal. Vibration not perceived at all is scored as absent, and vibration perceived initially but not at 5 seconds is scored as abnormal. The vibration test is reproducible and accurate test. It provides a quantitative assessment of DSPN and can document severe neuropathy, even in the presence of a normal result with the 10-g monofilament test [33]. The risk of foot ulcers is associated with diminished vibratory sensation and can be detected earlier and more accurately with the great toe vibration test. It is accepted that great toe vibration test should replace the 10-g monofilament test as the recommended technique for detection of DSPN [33].

d) Thermal sensory testing: Quantitatively testing of warm and cold is one of the tests currently being used for early detection of diabetic neuropathy such e. g. DSPN (This test is performed by using glass tubes filled with cold and hot water (thermal sensation, allodynia test, aftersensation test). Sensory loss and positive sensory signs (evoked and spontaneous pain, paresthesias) are recorded [22, 35]. Nowadays, pain and temperature sensation thresholds are measured by some devices for the evaluation of small nerve fiber dysfunction [36].

e) Pain-related evoked potentials: It is elicited by a nociceptive electrical stimulation of the skin and may contribute to the early detection of diabetic sensory neuropathy [37]. This method is one of the easiest and most reliable of the neurophysiological methods for assessing function of nociceptive pathways (mainly A delta fibers) [25].

f) Position sense: Sense of movement and position of toes and hand fingers were evaluated. Position sense was assessed at the interphalangeal joint of each great toe for a position change. The toe was held at both sides of proximal phalanx with one hand while using the other hand to move the distal phalanx up or down. After three trials of exam for each foot, the patient was asked to choose "up or down?" and the three responses per foot were scored as correct or incorrect [38].

4. Electromyography:

Nerve conduction studies (NCSs) are the most frequently used diagnostic tool for evaluation of any case of neuropathy in the patients with diabetes.

5. Corneal confocal microscopy:

It is a new sensitive non-invasive examination, which may be used to detect early small fiber nerve damage in patients with small nerve fiber neuropathy. Corneal nerve parameters are assessed and it allows quantification of corneal nerve morphology. Cornea nerve damage correlates with the severity of pain and autonomic neuropathy [39, 40].

6. Skin biopsy:

Nowadays it is included in the diagnostic work-up of patients with suspected small-fiber neuropathy [41]. This technique is used to show that small fibers degenerate early in the course of neuropathies associated with diabetes and it confirms the diagnosis when clinical and neurophysiologic examinations are not informative for diabetic neuropathy [42, 43]. A skin biopsy is also important to say about pain. Information from the literature suggest that a more serious loss of intraepidermal nerve fiber density increases the hazard to develop neuropathic pain, whereas intraepidermal nerve fiber density regeneration can be linked with a reduction in pain intensity [44]. Skin biopsy shows a diagnostic efficiency of 88.4%, clinical examination of 54.6% and quantitative sensory tests about 46.9% in patients with small fiber neuropathy [22, 35, 41].

7. Laser-Doppler-imager (LDI) flare technique:

LDI flare may represent a novel method for detecting early small-nerve-fiber changes in people with diabetes. The LDI flare easily detects small-fiber dysfunction before the occurrence of potentially irreversible structural loss of nerve fibers and can demonstrate abnormal C-fiber function in subjects with symptomatic DSPN in whom the results of conventional quantitative sensory tests are normal and in whom there is no significant reduction in nerve fiber density NFD [24]. In this non-invasive technique, axon reflex mediated vasodilatation in response to skin heating is measured and C-fiber dysfunction is detected in type 2 diabetes before small-fiber neuropathy can be discovered by other currently available noninvasive methods [24, 45]. Skin proximal to the first and second metatarsal heads on the dorsum is heated with a circular skin heater (diameter 1.0 cm; Moor Instruments) to 44°C for 20 min. An area of surrounding the heated skin was scanned with the LDI immediately after careful removal of the heater probe [24, 45]. On the flux image, the region of interest demarcated by the edge of the flare is drawn, and the area of the LDI flare is calculated and expressed in square centimeters [24].

8. Neuropathic pain assessment:

Although only some patients with nerve lesions develop neuropathic pain, it is a significant issue

because neuropathic pain is associated with poor quality of life and in that respect is no recognized objective gold standard for assessing neuropathic pain. Clinical assessment and diagnosis of neuropathic pain, a clinical history of pain and sensory exam are established to confirm nervous system involvement. Standardized screening tools which are often applied to distinguish and quantify neuropathy provide a full clinical record for follow-up of the patients with any case of neuropathy [46]. The diagnostic accuracy of neuropathic pain screening and assessment tools varies across patient populations. The DN4 may be more universally applicable than other creatures (e.g. The Leeds assessment of neuropathic symptoms and signs, PD-Q, and Neuropathic Pain Questionnaire) in general [47]. In DN4 scoring system, the presence of neuropathy symptoms (1 - Burning, 2 - Painful cold, 3 - Electric shocks, 4 - Tingling, 5.- Pins and needles, 6 - Numbness, 7 - Itching) and examination findings (1 - Hypoesthesia to touch, 2 - Hypoesthesia to prick, 3 - Allodynia by brushing) are evaluated. The total score is calculated as the sum of the 10 items and the cutoff value for the diagnosis of neuropathic pain is a total score of 4/10.

Classification and Diagnostic Work-up in Diabetic Neuropathies

A. Chronic symmetric neuropathies

Diabetic sensoriomotor polyneuropathy

The most common complication is peripheral neuropathy and distal symmetric polyneuropathy (DSPN) occurring in up to 50% of patients is the most common presentation of neuropathy in diabetes [48-50]. Chronic inflammatory demyelinating neuropathy which has similar sensory findings as seen in DSPN of diabetes is also more common in diabetic than in non-diabetic patients [51]. Typical DSPN is defined as a "chronic, symmetrical, length dependent sensorimotor polyneuropathy" [50, 52]. It is a commonly seen polyneuropathy with small and large fibre sensory, motor and autonomic involvement in various combinations. Diagnosis of DSPN is primarily clinical and needs a good history taking and neurological examination [50, 52]. A minimum of two abnormalities (from symptoms, signs, nerve conduction abnormalities, quantitative sensory tests, or quantitative autonomic tests) is needed in diagnosis [53]. In other words, documenting an abnormal sensory and motor examination of feet and great toe, and Aschille tendon reflex is sufficient to diagnose neuropathy with appropriate symptoms in a diabetic patient. Because of this reason, the use of monofilaments to assess touch-pressure sensation should not be forgotten as well as the Rydell-Seifert semi-quantitative tuning fork [54, 55]. New diagnostic techniques (including measurement of nerve fiber density using corneal confocal microscopy, and nociceptive evoked potentials) may contribute to the diagnostic work-up [53-56]. In the examination of the most serious complications such as foot ulcers, Charcot foot abnormalities and small injuries, they are checked visually [56]. To assess peripheral vascular disease as a possible cause of foot problems, peripheral pulses are checked as well.

Symptoms and signs: Sensory symptoms are more prominent, and the neuropathy symptoms start in the feet and spread proximally in a stocking glove distribution with increasing duration or severity of diabetes [57]. Both "positive" (tingling, burning, and other abnormal sensations) and "negative" (sensory loss, weakness, numbness, and unsteady gait) sensory symptoms may be seen [50, 52, 57, 58]. Small fibers are firstly effected and manifests with the impairment of pain, temperature and autonomic functions. Patients may report superficial burning pain or deep aching pain, tingling and numbness in their toes [11, 59]. They feel that their feet are persistently cold. Progression of sensory symptoms predisposes the patients to falls, development of foot ulcers and decreased quality of life [58]. Upwards to 50% of patients may experience neuropathic pain symptoms worse at night and disturb sleep [60]. What a pity, as up to 50% of the patients may be asymptomatic, a diagnosis may simply be made on examination and the patient presents with a painless foot ulcer in some cases [56, 60]. Small fiber neuropathies may be experienced even in the patients with impaired glucose tolerance and motor and reflex tests are normal during that stage [11, 48, 59]. Subsequently, the large nerve fibers are damaged and then gait disturbance due to impaired proprioception, mild distal weakness such as weakness of big toe and autonomic symptoms (pallor alternating with rubor, cyanosis, and mottling) may be seen [11, 57, 58]. Examination of the lower limb reveals distally located signs such as loss of pinprick, temperature, touch and vibration sense with reduced or absent ankle jerks and loss of great toe position sensation [60, 64]. Distal weakness occurs only in the most severe instances, so testing of the Achilles tendon reflex often indicating DSPN even in mild or asymptomatic cases is significant. In a typical DSPN

case, monophasic or fluctuating course and asymmetric or proximal symptoms as well as motor involvement may be seen. Acute neuropathic pain localized distally is another atypical characteristic of DSPN cases and pain may come in even prediabetes cases [61-63]. Any two of the following: neuropathy symptoms, decreased distal sensation, or decreased/absent ankle reflexes make DSPN diagnosis probable. For a definite diagnosis, it should be confirmed by abnormal nerve conduction study [64].

Differential diagnosis: Any variation of clinical findings such as acute, asymmetric, proximal, or motor involvement suggests an atypical neuropathy. In cases with sensory ataxia or profound distal upper and lower extremity weakness, an evaluation for other causes of neuropathy i.e. CIDP is recommended [65]. It should be also remembered that progression of DSPN is usually quite slow except the unusual cases of severe sensory and autonomic neuropathy that can occur in the first several months after the onset of Type 1 diabetes [65, 66]. The severe proprioceptive problem occurs when there is prominent large fiber involvement. These patients develop sensory ataxia and autonomic manifestations with impotence, bladder atony, and pupillary changes and thus have been called the pseudotabetic form of diabetic neuropathy. The presence of proprioception deficits and ataxia should lead to a search for other possible etiologies (syphilis, B12 deficiency, paraneoplastic or Sjogrens syndrome sensory neuropathy, CIDP) [66, 67]. Nevertheless, it is thought that the pseudosyringomyelic, pseudotabetic, and the early onset neuropathy described by Said et al. [66], all are severe variants of diabetic DSPN and probably not distinct forms of neuropathy [65-67]. Finding electrophysiologic changes that fulfill demyelinating criteria superimposed on axonal degeneration in a diabetic should be assessed with care. Likewise progressive polyradiculoneuropathy with a presentation similar to CIDP in diabetes may be seen, CIDP can develop in diabetic patients, too [67-69]. The clinical presentation of progressive poly-radiculoneuropathy is dominated by painful progressive motor weakness, with or without exacerbation of sensory symptoms. The weakness involves all limbs, but is often asymmetric. These symptoms and signs are important in differential diagnosis from classical DSPN. Electrophysiology of polyradiculoneuropathy may be predominantly axonal similar to DSPN, and CSF may show increased protein similar to CIDP cases [69]. Becuase nerve conduction studies and nerve biopsy are not helpfull in differential diagnosis, the clinical features of gradually progressive, usually painless, proximal and distal symmetric weakness and numbness in the arms and legs should be sufficient to distinguish CIDP from the typical symmetric and asymmetric diabetic neuropathies [65].

Screening tools: Utah Early Neuropathy Scale examing great toe dorsiflexion, pin sensation, great toe vibration and joint position, allodynia, and deep tendon reflexes is a sensitive and reproducible clinical measure of sensory and small-fiber nerve damage and may be useful in early diagnosis of diabetic neuropathy [70]. On the other hand, several examination scales focusing on large-fiber function have been risen to establish DSPN diagnosis.The Michigan Neuropathy Screening Instrument (MNSI) is a good screening tool for diabetic neuropathy and the Michigan Diabetic Neuropathy Score (MDNS) having higher concordance with nerve conduction studies provides a simple support to confirm this diagnosis [71, 72]. In the evaluation of neuropathic pain in diabetes, DN4 questionnaire or LANSS easily identifies potential patients with neuropathy, particularly by non-specialists, but it should be remembered that they cannot replace careful clinical judgement [73].

Electrophysiological tests: Nerve conduction studies (NCSs) are the most frequently used diagnostic tools for DSPN and used for confirmation. The main feature of DSPN is reduced distal lower extremity sensory nerve action potential amplitudes [74]. In early cases, nerve conduction velocity studies are usually normal when only small-diameter fibers are damaged. Although NCSs have limited utility in diagnosing small-fiber neuropathy in the early period of neuropathy related to diabetes, it is a reliable procedure to exclude any other etiology (superimposed nerve entrapments or inflammatory demyelinating polyneuropathies, neuropathies with asymmetrical symptoms or involvement of the upper limbs etc.) [52, 75]. Despite much study reports that the underlying process is axonal degeneration and/or demyelination, axonal degeneration is the primary process [76]. Follow-up changes in peroneal motor conduction velocity or sural sensory amplitude have often been used as primary progression measures in diabetic neuropathy trials because they are objective, repeatable, and continuously variable [52, 75-77].

Laboratory tests: Small fiber predominant

neuropathy presenting with pain and dyesthesias in the feet is usually an early manifestation of the typical DSPN in diabetes. To show decreased intra-epidermal nerve fiber density by Laser-evoked potentials and skin biopsy is very helpful because the clinical examination and nerve conduction studies may be normal in the early period [73, 78, 79]. Histological analysis of tissue taken from the distal part of the leg, within sural area 10 cm above the lateral malleolus in cases of suspected DSPN shows degenerated small-diameter sensory fibers. A low intraepidermal nerve fiber density below the fifth percentile is usually considered confirmatory for a diagnosis of early cases of DSPN [42, 43]. Screening laboratory tests providing the highest yield of abnormality are blood glucose, serum B12 with metabolites (methylmalonic acid with or without homocysteine) and serum protein immunofixation electrophoresis in the patients with DSPN. Metformin interferes with the absorption of cobalamin and may contribute to vitamin deficiency. Vitamin B12 deficiency should be certainly evaluated because it can lead to progressive deterioration of nerve tissue frequently misdiagnosed as diabetic peripheral neuropathy [80-82]. On the other hand, functional deficiency of vitamin B12 (ie, normal cobalamin but elevated MMA levels) is another problem with a tripling of neuropathy frequency in type 2 diabetes patients (regardless of metformin use) [80-82]. Infections (e.g., Lyme disease, HIV, Hepatitis), vasculitis, alcohol use, cancer and related paraneoplastic syndromes should also be assessed in order to exclude other etiologies of polyneuropathy. Furthermore, diabetic neuropathy may be associated with demyelination, and diabetes mellitus, multifocal motor neuropathy and CIDP may have elevated CSF protein [83]. Because of this diagnostic problem, we should consider all possible causes of neuropathy in order to properly treat the diabetes patients [48]. Genetic testing may be considered in patients only with a history of hereditary neuropathy and cryptogenic polyneuropathy who exhibit a hereditary neuropathy phenotype [84].

B. Transient symmetric neuropathies (Acute, painful, sensory predominant)

1. Diabetic neuropathic cachexia (DNC)

It is an uncommon syndrome and symmetric sensory peripheral neuropathy is associated with "weight loss up to 60% of total body weight, symmetric painful dysesthesias over the limbs and trunk which is typically affecting trunk" [48, 85, 86]. It is not associated with weakness similar to DSPN. Patients may experience neuropathic pains tending to be worse at night or during periods of relaxation. The patients complain very severe pain, but sensory impairment associated with the DNC is usually minimal. The pain resolves with weight gain. Depression is one of the hallmarks of the syndrome [48]. The DNC is reversible with adequate diabetic control over weeks to months, unlike other symmetric diabetic neuropathies. Most reported patients are adult males, ordinarily in the sixth or seventh decades of lifetime. The DNC can occur in both types of diabetes patients and are associated with poor glucose control [48, 55, 56].

2. Hyperglycemic neuropathy

It is likewise a secret and mostly transient state. Hyperglycemic neuropathy can ocur at the time of diagnosis or may follow an episode of ketotic coma, and the symptoms rapidly subside once the diabetes is controlled. Distally located sensory paresthesias and pain have been declared but not proved to be due to hyperglycemia in these patients. The relationship between hyperglycemia and neuropathy is still not definite because the neuropathy can regress in these patients despite poor glucose control [48].

3. Insulin neuritis

The insulin neuritis is usually characterized by symmetric neuropathy with acute onset of severe distal limb pain and autonomic dysfunction in relation to intensive glycemic control following the institution of insulin or intake of oral hypoglycemic agents (predominantly small-fiber neuropathy manifesting with pain and paresthesia) [48, 87-89]. Typical cases occur after several episodes of protracted hypoglycemia with mostly insulinoma [48]. A classic patient may complain of distal paresthesia similar to DSPN. Although insulin neuritis involves the upper extremities more than the lower extremities, footdrop is also common [48]. Neuropathic symptoms such as hyperalgesia and allodynia which are refractory to medications persist for weeks or months and regress after control of hypoglycemia [47, 48].

C. Transient asymmetric neuropathies (Acute, painful, sensory-motor type)

1. Diabetic radiculoplexus neuropathies

They are acute or subacute forms of neuropathy and presents commonly with abrupt onset of pain followed by weakness and primarily affect patients

with mild diabetes [1]. The symptoms progress, then stabilizes and gradually improve over a few weeks. There are three types of radiculoplexus neuropathy named according to location.

a- Cervical radiculoplexus neuropathy: It is a very uncommon form of the radiculoplexus neuropathy.

b- Thoracic radiculoneuropathy: It typically presents with abrupt onset severe pain (burning, stabbing or belt like) with contact hyperesthesia along the thoracic spine, chest, rib cage or upper abdominal wall. Popping out of the abdominal wall may come because of muscle weakness. Truncal radiculoneuropathy can be symmetrical and involve multiple dermatomes, so it may be confused with intra-abdominal or thoracic disease or herpes zoster [1, 4, 90]. Needle EMG findings include fibrillations in the paraspinous or abdominal wall muscles [65].

3- Lumbar radiculoplexopathy: It causes the most morbidityand known by different names, including diabetic amyotrophy, Bruns-Garland syndrome, diabetic mononeuritis multiplex, diabetic polyradiculopathy, proximal diabetic neuropathy, and others [1, 90]. Motor involvement is predominant and weakness and wasting in the thigh are seen after unilateral severe pain to be more severe at night in the anterior thigh, buttock, lumber area or knee. The distal muscles of the leg are rarely involved. Knee jerk is absent [1, 90].

2. Diabetic mononeuritis multiplex

Mononeuritis multiplex (MNM) is a syndrome of painful, unilateral or asymmetrical, asynchronous sensory and motor multiple neuropathies at least 2 separate nerve areas [90, 92]. The areas affected by MNM depend on the underlying cause of the condition. This case of neuropathy tends to occur in older patients with relatively mild cases of diabetes. After the abrupt onset of pathology because of occlusion of the vasa nervosum in one nerve, multiple nerves are affected in a random distribution. As the condition worsens, it becomes less multifocal and more symmetrical. Individuals with diabetes typically present with acute onset of severe, unilateral thigh pain that is followed rapidly by weakness and atrophy of the anterior thigh muscles and loss of the knee reflex (see diabetic amyotrophy) [1, 90]. As mononeuropathy multiplex or multifocal neuropathy can occur with diabetes mellitus, it can be also associated with many different conditions such as infections, rheumatological disorders, cancer-related conditions and hematological conditions. In electrodiagnostic studies, a reduction in the motor and sensory nerve action potential amplitudes and minimal alterations in nerve conduction velocity are seen in the patients with MNM [92]. In diabetic multifocal motor neuropathy, mild inflammatory infiltrates and loss of axons may be observed in addition to conduction bloks [48, 92]. The needle electrode examination findings can vary and acute and chronic denervation may be seen depending on the severity and time course of the disorder. Nerve biopsy may be required as part of the diagnostic evaluation in cases with vasculitis which is usually treatable with immunosuppressive medications [48]. Any condition with asymmetrical polyneuropathy is considered in the differential diagnosis of MNM. As last words, the usual causes need to be pursued (vasculitis, infections, and hereditary) if a diabetic patient develops a true MNM [65].

3. Cranial neuropathy

The upper cranial nerves are affected (third, sixth, rarely fourth and seventh in order of frequency) [48, 93]. Diabetic third nerve palsy presents acute ophthalmoplegia with abrupt onset of pain behind or above the eye, followed by double vision, unilateral ptosis, paresis of the oculomotor innervated muscles and sparing of the pupil which is the hallmark of diabetic third nevre palsy [48, 93]. Cranial neuropathy is attributed to presumed microvascular ischemia of nerve [93, 94]. If there is pupillary involvement or absence of pain, there is concomitant autonomic nerve involvement or aneurysm which must be excluded by neuroimaging [93, 94]. Typically, cranial neuropathies in diabetes mellitus improve and settle within 2 to 3 months [48, 93, 94]. Imaging studies may be required to rule out stroke in some shells. Nevertheless, history alone without additional testing is sufficient in the bulk of these patients [65]. Pupillomotor function impairment (e.g., decreased diameter of dark adapted pupil) and Argyll-Robertson pupil abnormality can be seen as a result of autonomic dysfunction [1].

4. Mononeuropathies

Mononeuropathies often emerge during periods of transition in the diabetic illness, or when there has been rapid weight loss and nerve entrapment seems to be more common than nerve infarction [89, 90]. The nerves most frequently affected are the femoral, sciatic and peroneal ones in that order. Patients with diabetes are also at increased risk of entrapment

mononeuropathy. A higher prevalence of compressive neuropathies of median or ulnar nerves exists in patients with diabetes in comparison to the general population [48]. The second most commonly seen neuropathy in the patients with diabetes is carpal tunnel syndrome, which is 3 times more common in comparison to normal population [6, 89, 90]. The mononeuropathies are easily can be diagnosed using electromyography.

D. Diabetic autonomic neuropathy (DAN)

All organs are susceptible to autonomic dysfunction because vasomotor, visceromotor, and sensory nerve fibers innervate every organ. DAN is a widespread disorder and may be manifested by dysfunction of one or more organ systems. DAN typically affects both parasympathetic and sympathetic parts of the autonomic nervous system, so it results in cardiovascular, gastrointestinal, urination, sweating, pupils, and metabolic disturbances [48, 95]. Clinical symptoms of DAN generally do not occur in early phases of diabetes and it manifests first in the vagus nerve which is the longest of the ANS nerves. The cardinal signs of DAN are orthostatic/postural hypotension, orthostatic bradycardia and orthostatic tachycardia, resting tachycardia, exercise intolerance, decreased hypoxia-induced respiratory drive, impaired heart rate variability, heart rate unresponsiveness to respiration and abnormal blood pressure regulation. [48, 96]. Because of all these problems mentioned above, DAN may be linked to increased incidence of asymptomatic ischemia, myocardial infarction, and decreased rate of survival after myocardial infarction [95, 96]. Age, duration of diabetes, obesity, smoking, and poor glycemic control are risk factors increasing DAN prevalence [96]. What a pity, most of the cases with DAN have subclinical forms without the presence of signs and symptoms and they are detectable only by tests [48, 96]. Subclinical forms of DAN may be detected within 1 year of diagnosis with type 2 diabetes and within 2 years of diagnosis with type 1 diabetes [48, 96, 97]. Although cardiovascular autonomic neuropathy (CAN) is the most clinically important and easily-studied form of DAN, DAN involving other organ systems should also be viewed in the optimal care of patients with diabetes.

1. Cardiovascular Autonomic Neuropathy (CAN)

There is a problem of autonomic control of the cardiovascular system in CAN and the earliest manifestations of autonomic neuropathy in diabetes tend to be associated with parasympathetic denervation because of vagal involvement which represents the longest fibers [48, 95]. Reduced heart rate variation is the earliest indicator of CAN and it increases mortality rate because of myocardial infarction or malignant arrhythmia [95, 97].

Symptoms and signs: Unexplained tachycardia or bradycardia, orthostatic hypotension and poor exercise tolerance should be assessed for the presence of CAN [48].

Tests: Nowadays it is possible to objectively identify early stages of CAN with the usage of different autonomic function tests. Screening tests should be performed particularly in the patients with DSPN, poor glycemic control, cardiovascular risk factors, and vascular diabetic complications [52]. In comparison of a variety of simple, validated, and noninvasive tests (e.g., Valsalva maneuver, Handgrip test, Heart rate response to deep breathing and standing, and blood pressure response to standing), valsalva maneuver and heart rate variation tests are more common than others [52, 95-97].

a- Exercise intolerance: When you begin to exercise, your heart rate increases rapidly to the level necessary to meet the metabolic needs of increased activity. Diabetic patients who are likely to have CAN show a reduced response in heart rate and blood pressure during exercise and it is called as exercise intolerance [95]. Degrees of exercise intolerance can vary and it can exhibit itself after the mildest exertions or require more sustained effort to be recognized. Fatigue, cyanosis, muscle cramps, insufficient heart rate, blood pressure changes and depression are the most common signs of exercise intolerance [95-96].

b- Orthostatic hypotension: Orthostatic hypotension is defined by consensus as a fall in blood pressure of at least 20 mmHg systolic and/or 10 mmHg diastolic within three minutes in an upright position. Blood pressure and heart rate are measured after 5 min supine and at 1 and 3 min after standing; patients unable to stand may be assessed while sitting upright. Hypotension without a compensatory increase in heart rate (< 10 beats/min) suggests autonomic impairment. Faintness, light-headedness, dizziness, confusion, or blurred vision with postural changes is seen. Symptoms occur within seconds to a few minutes of standing and resolve rapidly on lying down. Exercise or a heavy meal may exacerbate symptoms [96]. Questionnaires may be used to investigate orthostatic symptoms and their severity in

dysautonomic conditions [1, 96].

c- Heart rate variability (HRV): Heart rate variability (HRV) is the physiological phenomen on of variation in the time interval between heartbeats. When the autonomic system is intact, heart rate increases in response to inspiration. Autonomic function can be evaluated with bedside cardiac monitoring. The autonomic evaluation consisted of measuring HRV for 30 min in supine position in absence of any physical, sensory, or pharmacological stimuli. R-R variation between supine and standing position is measured. If R-R supine/R-R standing is lower than 1.03, it is accepted as abnormal [95, 98, 99]. Patients with abnormal R-R intervals or with autonomic symptoms or signs require further evaluation for diabetes [95, 98, 99].

2. Gastrointestinal Autonomic Neuropathy (GAN)

It is difficult to evaluate gastrointestinal autonomic function in humans. Gastroparesis is the most debilitating complication of GAN [1, 99]. The diagnosis of GAN is often one of exclusion, because many of the gastrointestinal symptoms related to sympathetic and parasympathetic nervous systems may be seen (52, 96]. GAN symptoms are associatedwith poorglycemic control, malnutrition, abnormal postprandial regulation of blood pressure, poor quality of life, and a high rate of hospitalization in the patients [52, 100].

Symptoms and signs: Nausea, bloating, dysphagia and chest pain secondary to esophageal dysmotility, constipation and abdominal pain secondary to gastroparesis diabeticorum, diarrhea, and fecal incontinence may be seen. Up to 75% of patients with diabetes can experience these symptoms mentioned [100].

Tests: Objective gastric emptying measurement may be used for the diagnosis of gastroparesis. To quantify gastrointestinal symptoms, the Diabetes Bowel Symptom Questionnaire is applicable [52].

3. Erectile Dysfunction

Erectile dysfunction or impotence is the inability to develop or maintain an erection of the penis during sexual activity. The most important organic causes are aging (>60 years of age), cardiovascular disease and diabetes. Adverse or unanticipated drug interactions, obesity, sex (hypogonadism and prolactinoma), medical causes (groin hernia, trauma from prostatectomy surgery, multiple sclerosis, kidney failure), drugs (SSRIs and nicotine), alcohol use, tobacco use and psychosocial causes (anxiety, mental disorders, stress) are potential risks [52]. Erectile dysfunction affects 30-40% of diabetic men and is an early marker of cardiovascular risk because of endothelial dysfunction [1]. The use of validated questionnaires such as the international index of erectile function or quality of erection questionnaire is the most appropriate method to evaluate the erectile dysfunction symptoms [52, 101, 102]. In practice, the problem is likely to be physiological if the patient never has an erection; it could be physiological or psychological if sometimes (however rarely). Duplex ultrasound exam, the bulbocavernosus reflex test, nocturnal penile tumescence assessment and cavernosography are the most commonly used investigations to determine erectile dysfunction. Specific testing may be recommended in patients not responding to phosphodiesterase-5 inhibitor which is the first effective oral treatment available for erectile dysfunction [52]. In female with genitourinary autonomic neuropathy, loss of lubrication is seen [95, 99]. Retrograde ejaculation due to neuropathy of the bladder sphincter is another complication of diabetes [99].

4. Diabetic Sudomotor Dysfunction

Loss of thermoregulatory sweating in a ‘glove and stocking’distribution that can extend to the upper parts of thelimbs and the anterior abdomen, conforms the length dependency of diabetic neuropathy. Not only anhidrosis but also hyperhidrosis can be seen as a sign of DAN [1, 103]. Abnormal production of sweatthat appears over the upper part of the body after consumption of even non-spicy foods, occurs in occasionalcases [103]. The quantitative sudomotor axon reflex test is capable of detecting distal small-fiber polyneuropathy with a sensitivity of 75% [52].

5. Diabetic Bladder Dysfunction

In patients with diabetes and bladder dysfunction, sensory and autonomic nerve fibers areaffected [104]. Patients often remain asymptomatic in early stagesand impaired bladder sensation is usually the first manifestation of lower urinary tract involvement [1]. The usual symptoms are straining, hesitation and weakness of stream [52, 104]. Impaired sensation of bladder fullness leads to overstretched bladder, reduced bladder contractility, increased residual urine and impaireduroflow in the patients with diabetic bladder dysfunction [104].

Conclusion

DSPN and carpal tunnel syndrome are commonly experienced problems in the patients with diabetes. Clinical evaluation and use of scoring systems for the evaluation of diabetic polyneuropathy is an important step to get a correct diagnosis. NCSs confirm the diagnosis of DSPN, entrapment neuropathies, MNM or radiculoplexopathy. SWME (1-10 g), vibrating test and ankle reflex can be used confidently for annual screening of diabetic neuropathy in diabetic patients. By using both the SWME and vibration tests, it can be differentiated non-diabetic control subjects from subjects with diabetes, as well as subjects with diabetes with and without neuropathy. The DN4 questionnaire is utilized for the evaluation of neuropathic pain. The primary advantage of this screening instrument which is comfortable to use and delivers a good diagnostic performance is to identify potential patients with neuropathy, especially by non-specialists. Because neuropathy at the stage in which only small characters are affected can be annulled, it is important to diagnose DSPN in early phases. Skin biopsy taken from the dermatomal area of the sural nerve, laser-doppler-imager flare technique, corneal confocal microscopyare used to assess small fiber dysfunction in these patients with peripheral neuropathy, with the aim to demonstrate specific findings for diagnosing small fiber neuropathy.

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Personnel radiation dose assessment using a novel dosimeter in the department of radiology and dentistry in a medical facility in Delta State, South-South Nigeria: our experience in the last 4 years

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ABSTRACT

Objectives. Large percentages of X-ray facilities in Nigeria do not use radiation monitoring device; a few percentage that use them do not evaluate or carryout out assessment programs to ascertain the detriment to occupationally exposed workers. This study was aimed at evaluating dose reports from 2013 to 2016 for personnel who operate radiation facilities and those that work within radiation field during certain X-ray procedures/examinations in the department of radiology and dentistry respectively; to ascertain if there is correlation between personnel dose and workload in both department and to determine if dose records are within acceptable limit recommended by the International Atomic Energy Agency (IAEA) safety series. ***Methods.*** Direct Ion Storage (DIS) dosimeter was used for a total of 35 occupationally exposed personnel who work in the department of radiology and dentistry. The DIS dosimeter was read every two months and results were authomatically saved on the instadose™ platform. ***Results.*** The mean (total) dose in radiology department for the first, second, third and fourth year was 0.17 ± 0.08 (3.52) mSv, 0.08 ± 0.03 (0.77) mSv, 0.07 ± 0.04 (0.72) mSv and 0.07 ± 0.05 (0.55) mSv and in Dentistry was 0.08 ± 0.02 (0.73) mSv, 0.05 ± 0.02 (0.42) mSv, 0.05 ± 0.02 (0.24) mSv and 0.07 ± 0.04 (0.34) mSv; respectively. There was significant difference in mean personnel dose from 2013-2016 in Radiology (p=0.028) and in Dentistry Department (p=0.004). Correlation of workload and personnel dose in Radiology (p=0.240) and Dentistry Department (p=0.765) wasn't significant. There was no correlation in mean dose between both department (p=0.256). **Conclusion.** Overall mean dose in both department for occupationally exposed personnel were below IAEA annual dose limit of 20 mSv averaged over a period of 5 consecutive years. Dose reports of personnel in both department reduced as the year progressed due to radiation safety awareness.

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Keywords: Direct ion storage dosimeter, ion-chamber, panoramic, cephalometric, workload

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Introduction

In late 1895, X-ray was discovered by Wilhelm Conrad Röntgen; a professor of Physics at the University of Würzburg Germany, ever since this discovery, Medicine and other fields have benefited immensely on its use [1-3]. The use of radiation for medical diagnostic examinations has contributed approximately over 95% of man-made radiation exposure and is only exceeded by natural background as a source of exposure to the world's population [4, 5]. The effect of exposure to ionising radiation became evident only a few years after its discovery. This scenario points to the fact that sites/environment where these machines are used may be under threat of secondary (scatter and leakage) radiation if no safety standards and precautions are followed [6-8]. This has made the International community to look into how this "useful but dangerous particle can be used effectively". In the same vein, several International bodies have been established with several roles on how well to manage ionizing radiations; one of such is the International Atomic Energy Agency (IAEA) [9]. Today, it is widely used in diagnosis and treatment of malignancy like cancer and other tumours. One major casualty of eventual fallout of radiation is the occupationally exposed personnel who spend their life time working within radiation facilities [10-12]. In Nigeria today there are over 4,000 X-ray machines in use [13]. The Nigerian Nuclear Regulatory Authority (NNRA) which is the country's national body is saddled with the responsibility of regulating radiological protection and nuclear safety so as to ensure the protection of life, health, property and the environment from the harmful effect of ionizing radiation [14].

In Nigeria, studies have shown that many hospital-based Radiology/Dental department and privately owned diagnostic X-ray centres do not use radiation monitoring devices; a few that use them do not evaluate their reports/records over a long period of time [15, 16].

Several devices are currently in use for personnel dose measurement, such as the P-Channel Metal-Oxide Semiconductor (pMOS) with Radiation-Sensing Field Effect Transistor (RADFET) dosimeter [17, 18], Direct Ion Storage (DIS) dosimeters [19-23], Thermoluminescent dosimeter (TLD) [24] and Optically Stimulated Luminescent (OSL) dosimeters [25]. Currently in Nigeria only a few X-ray facility use OSL, majority use TLD [14]. More recently, the limitations of TLDs in Nigeria were attributed to problem arising from distances between dosimeter providers and end users, activation of heat while transporting them and the inability of end-users to get readouts at anytime they so desire. The above mentioned problems gave rise to the use of DIS dosimeter which can be read with a mobile device at anytime and anywhere.

This study would focus only on occupational exposed personnel who are assigned a new type of dosimeter called the DIS dosimeter. Also, the study will evaluate dose records of occupationally exposed personnel and those who occasionally work close to X-ray facilities both in Radiology and Dentistry Department and will determine if personnel radiation dose have direct correlation with workload which is a function of the X-ray output per week at a well defined point in each department and to determine if personnel mean effective doses are within acceptable dose limits, specified in the IAEA Safety Series and International Commission on Radiation Units and Measurements (ICRU) publication 60 and 103 [6, 7, 26, 27].

Methods

This research was a retrospective study, which was carried out from 2013 to 2016. The personnel involved in this study were permanent staffs of the hospital. In Radiology Department they include: Radiologist, Radiographers, Imaging Technicians, Health Attendants/Nurses, Porters and a Medical Physicist. Exempted from this study were Intern Radiographers, undergraduate Clinical Radiography student, undergraduate Imaging Technicians, cleaners and clerical officers. In the same vein, personnel involved in this study from Dentistry Department were: Dental Surgeons and Dental Technicians. Exempted were undergraduate Dental Technicians. Intern and student were also monitored but their dose data/records were not included in this study. The reason for this exemption was because of their short stay in both departments. Ethical approval was granted by the institution where the research took place.

The material used for this study was Forty pieces of Direct Ion Storage Dosimeter (25 pieces of it was used in Radiology and 15 pieces was used in Dentistry department). The DIS dosimeter can be activated and assigned to a user. It can also be deactivated if a user no longer works with the X-ray facility and can be reassigned to another user.

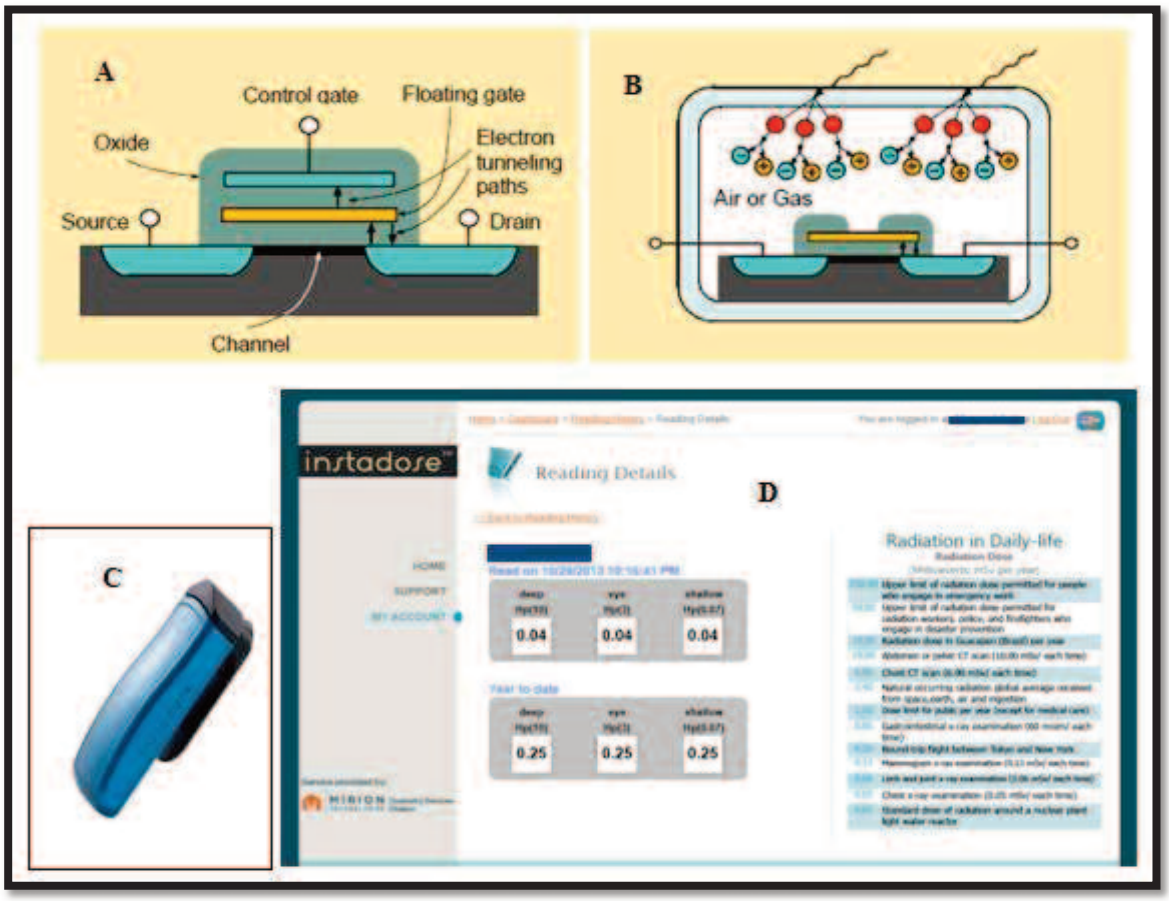

Figure 1. (A) Analog EEPROM memory cell, (B) DIS memory cell with the formation of a conductive wall (ion chamber), (C) DIS Dosimeter, (D) instadose™ Readout platform

The Radiological equipment used were two Conventional (both floor mounted) X-ray machine with maximum voltage/current of 125kVp/500mAs and 150kVp/630mAs respectively, one Fluoroscopy X-ray machine with maximum voltage/current of 150kVp/800mAs and one Mammography X-ray machine with maximum voltage/current of 35kVp/500mAs. The dental equipment used were five wall mounted intra-oral (IO) X-ray machine each of anode voltage and current of 70kVp and 7mA, one Panoramic/Cephalometric X-ray machine with maximum anode voltage and current of 90kVp and 10mA and exposure timer of 13 sec for Panoramic and 15sec for Cephalometric . The mentioned equipment was used throughout the study.

The principle of operation of the Direct Ion Storage (DIS) dosimeter is a combination of a hybrid of ion chamber and Floating Gate Metal-Oxide-Semiconductor Field-Effect Transistors (FGMOSFETs). In the DIS memory cell, the oxide layer surrounding the floating gate has an opening allowing the surface of the floating gate to be in direct contact with the surrounding air (or any other gas). The ionising radiation incident in the air or gas produces electron-ion pairs which is effectively formed between the wall and the floating gate by surrounding the entire structure with a conductive wall (ion chamber) with extremely high mobility and in case there is an electric field surrounding the floating gate, these charge carriers will be transferred efficiently to the gate before any recombination occurs [Figure 1A]. The DIS dosimeter contains an Analog-Electrical Erasable and Programmable Read Only Memory (EEPROM) cell [Figure 1B]. The charge in the floating gate can be set to a predetermined level by tunnelling electrons through the oxide layer. The charge is then stored permanently in the gate due to the fact that in the normal operating temperature range the electrons have a very low probability of exceeding the energy barriers in the metal-oxide and oxide-silicon interfaces carriers, of which Na-ions are usually the most dominant. Today it is possible to manufacture memory cells that are capable of retaining a stored charge for hundreds of years. Reading the stored information is carried out by measuring the channel conductivity of the transistor without disturbing the stored charge.

The DIS dosimeter has the capacity to measure

Table 1. Radiology mean personnel dose for year 2013 & 2014

Assigned user	Group	X-ray Equipment used	$H_P(10)$ Deep dose (mSv)	$H_P(0.07)$ Shallow dose (mSv)	$H_P(3)$ Eye dose (mSv)	Body Region
Year 2013						
R_1	Radiographer	C	0.10	0.10	0.10	Torso
R_2	Radiographer	C/M	0.10	0.10	0.10	Torso
R_3	Radiographer	C/M/F	0.17	0.17	0.17	Torso
R_4	Radiographer	C/M/F	0.13	0.13	0.13	Torso
R_5	Radiographer	C/M/F	0.20	0.20	0.20	Torso
R_6	Radiographer	C/F	0.17	0.17	0.17	Torso
R_7	Radiographer	C/F	0.18	0.18	0.18	Torso
R_8	Radiographer	C/F	0.12	0.12	0.12	Torso
R_9	Radiographer	C/F	0.15	0.15	0.15	Torso
R_{10}	Radiographer	C/F	0.11	0.11	0.11	Torso
R_{11}	Radiographer	C/F	0.15	0.15	0.15	Torso
R_{12}	Porter	***	0.09	0.09	0.09	Torso
R_{13}	Health. A/Nurse	C/M/F	0.07	0.07	0.07	Torso
R_{14}	Technicians	***	0.05	0.05	0.05	Torso
R_{15}	Technicians	***	0.10	0.10	0.10	Torso
R_{16}	Technicians	***	0.08	0.08	0.08	Torso
R_{17}	Technicians	***	0.09	0.09	0.09	Torso
R_{18}	Technicians	***	0.07	0.07	0.07	Torso
R_{19}	Radiologist	C/F	0.44	0.44	0.44	Torso
R_{20}	Radiologist	C/F	0.45	0.45	0.45	Torso
R_{21}	Radiologist	C/F	0.50	0.50	0.50	Torso
Year 2014						
R_1	Radiographer	C	0.07	0.07	0.07	Torso
R_2	Radiographer	C/M/F	0.04	0.04	0.04	Torso
R_3	Radiographer	C/F	0.09	0.09	0.09	Torso
R_4	Radiographer	C/M/F	0.10	0.10	0.10	Torso
R_5	Radiographer	C/M/F	0.09	0.09	0.09	Torso
R_6	Radiographer	C/F	0.05	0.05	0.05	Torso
R_7	Porter	***	0.04	0.04	0.04	Torso
R_8	Technicians	***	0.06	0.06	0.06	Torso
R_9	Radiologist	C/F	0.12	0.12	0.12	Torso
R_{10}	Radiologist	C/F	0.11	0.11	0.11	Torso

*** Personnel proximal to the facility, C/M/F = Conventional/Mammographic/Fluoroscopic, A = Attendant

deep, shallow and lens dose. Measurements at the deep dose equivalent [HP (10)] are a concept that applies to external whole body radiation. It is the dose equivalent at a tissue depth of 1 centimetre. This quantity is usually determined using a "whole body" dosimeter. It does not apply to weakly penetrating radiation such as alpha particles or low-energy electrons. Also, the shallow dose equivalent [HP (0.07)] applies to external exposure of the skin of the whole body or the skin of an extremity. It is the dose equivalent just below the cornified layer of the skin at a tissue depth of 0.007 centimetre averaged over an area of 10 square centimetres. Thirdly, the lens or eye dose equivalent [HP (3)] applies to external exposure to the lens of the eye. It is the dose equivalent at a tissue depth of 0.3 centimetres. This quantity is usually determined using a "whole body" dosimeter worn at or near the torso level. It does not apply to weakly penetrating radiation such as alpha particles or low-energy electrons. The DIS minimum reportable dose is 3 rem [0.03 millisievert (mSv)] and 1rem (0.01 mSv) upon reset, with low limit of detection of 1 rem (0.01 mSv). The useful dose range is 0.01 mSv-5 Sv, with energy response of 5 keV-6 MeV [Figure 1C&1D]. The algorithm of the instadose^{TM} platform will only compute dose whenever equivalent dose is 0.03 mSv and will record “below reportable dose (BRD)” which is clearly shown on the readout of the instadose^{TM} platform whenever dose is < 0.03 mSv. The X-ray machine used by radiographer (C/F/M) and

Table 2. Radiology mean personnel dose for year 2015 & 2016

Assigned user	Group	X-ray Equipment used	H_P (10) Deep dose (mSv)	H_P (0.07) Shallow dose (mSv)	H_P (3) Eye dose (mSv)	Body region
Year 2015						
R_1	Radiographer	C/M/F	0.04	0.04	0.04	Torso
R_2	Radiographer	C/F	0.04	0.04	0.04	Torso
R_3	Radiographer	C/M/F	0.03	0.03	0.03	Torso
R_4	Radiographer	C/M/F	0.08	0.08	0.08	Torso
R_5	Radiographer	C/F	0.10	0.10	0.10	Torso
R_6	Radiographer	C/F	0.03	0.03	0.03	Torso
R_7	Health. A/Nurse	C/M/F	0.04	0.04	0.04	Torso
R_8	Technicians	***	0.03	0.03	0.03	Torso
R_9	Technicians	***	0.09	0.09	0.09	Torso
R_{10}	Radiologist	C/F	0.10	0.10	0.10	Torso
R_{11}	Radiologist	C/F	0.14	0.14	0.14	Torso
Year 2016						
R_1	Radiographer	C/M/F	0.06	0.06	0.06	Torso
R_2	Radiographer	C/M	0.03	0.03	0.03	Torso
R_3	Radiographer	C/F	0.15	0.15	0.15	Torso
R_4	Radiographer	C/M/F	0.03	0.03	0.03	Torso
R_5	Radiographer	C/M/F	0.04	0.04	0.04	Torso
R_6	Technicians	***	0.04	0.04	0.04	Torso
R_7	Radiologist	C/F	0.12	0.12	0.12	Torso
R_8	Radiologist	C/F	0.08	0.08	0.08	Torso

*** Personnel proximal to the facility, C/M/F = Conventional/ Mammographic/ Fluoroscopic, A = Attendant

technicians (C/P/IO) were indicated in the tables and personnel categorized as those that work proximal to facility operators (radiographer and dental technicians) were left empty [Table 1, 2 and 3].

Statistical Analysis

Data analysis was done using Microsoft Excel and SPSS Version 16.0 Software. A p value <0.05 was considered to be statistically significant.

Results

The mean/total dose in Radiology Department in 2013 was 0.17± 0.08 (3.52) mSv, 2014 was 0.08± 0.03 (0.77) mSv, 2015 was 0.07± 0.04 (0.72) mSv and 2016 was 0.07± 0.05 (0.55) mSv. The highest readout was noticed from the Radiologist unit (0.5 mSv) [Tables 1 and 2]. One-Sample T Test in Radiology department show that in 2013 the mean dose report among personnel had statistically significant difference (p=0.000), this was also similar in 2014 (p=0.000) [Table 1], 2015 (p=0.000) and 2016 (p=0.003) [Table 2]; respectively. Personnel mean dose from the first year of study (2013) to the fourth year of study (2016) showed that there was significant difference in dose value (p=0.028).

The mean/total dose in Dentistry Department in 2013 was 0.08± 0.02 (0.73) mSv with the highest readout from the Dental Technician, 2014 was 0.05± 0.02 (0.42) mSv with the highest readout from the Dental Surgeon, 2015 was 0.05± 0.02 (0.24) mSv with the highest readout from the Dental Technician and 2016 was 0.07± 0.04 (0.34) mSv with the highest readout from the Dental Surgeon (Table 3). In the same vein, One-Sample T Test in Dentistry department show that in 2013 the mean dose among personnel had statistically significant difference (p=0.000), this was also similar in 2014 (p=0.002), 2015 (p=0.003) and 2016 (p=0.012) (Table 3); respectively. Similarly, personnel mean dose from the first year of study (2013) to the fourth year of study (2016) showed that there was generally significant difference in dose value (p=0.004)

The average number of patient per week/workload in Radiology Department in 2103 was 187/340mA–min/Wk, 2014 was 159/290mA–min/Wk, 2015 was 185/328mA–min/Wk and 2016 was 160/242mA–

Table 3. Mean dose from 2013-2016 in the dental department using Panoramic, Cephalometric and Intra-Oral X-ray machine

Assigned user	Group	X-ray Equipment used	$H_P(10)$ Deep dose (mSv)	$H_P(0.07)$ Shallow dose (mSv)	$H_P(3)$ Eye dose (mSv)	Body region
Year 2013						
R1	Dental technician	C/P/IO	0.05	0.05	0.05	Torso
R2	Dental technician	C/P/IO	0.08	0.08	0.08	Torso
R3	Dental technician	C/P/IO	0.05	0.05	0.05	Torso
R4	Dental technician	C/P/IO	0.12	0.12	0.12	Torso
R5	Dental technician	C/P/IO	0.08	0.08	0.08	Torso
R6	Dental technician	C/P/IO	0.08	0.08	0.08	Torso
R7	Dental technician	C/P/IO	0.07	0.07	0.07	Torso
R8	Dental Surgeon	***	0.10	0.10	0.10	Torso
R9	Dental Surgeon	***	0.10	0.10	0.10	Torso
Year 2014						
R1	Dental technician	C/P/IO	0.04	0.04	0.04	Torso
R2	Dental technician	C/P/IO	0.03	0.03	0.03	Torso
R3	Dental technician	C/P/IO	0.03	0.03	0.03	Torso
R4	Dental technician	C/P/IO	0.04	0.04	0.04	Torso
R5	Dental technician	C/P/IO	0.06	0.06	0.06	Torso
R6	Dental technician	C/P/IO	0.05	0.05	0.05	Torso
R7	Dental Surgeon	***	0.10	0.10	0.10	Torso
R8	Dental Surgeon	***	0.07	0.07	0.07	Torso
Year 2015						
R1	Dental technician	C/P/IO	0.04	0.04	0.04	Torso
R2	Dental technician	C/P/IO	0.07	0.07	0.07	Torso
R3	Dental technician	C/P/IO	0.06	0.06	0.06	Torso
R4	Dental technician	C/P/IO	0.03	0.03	0.03	Torso
R5	Dental Surgeon	***	0.04	0.04	0.04	Torso
Year 2016						
R1	Dental technician	C/P/IO	0.04	0.04	0.04	Torso
R2	Dental technician	C/P/IO	0.03	0.03	0.03	Torso
R3	Dental technician	C/P/IO	0.12	0.12	0.12	Torso
R4	Dental Surgeon	***	0.05	0.05	0.05	Torso
R5	Dental Surgeon	***	0.10	0.10	0.10	Torso

P= Panoramic, C= Cephalometric, IO = Intra-Oral, *** Personnel proximal to the facility

min/Wk, with 2013 having the highest number of patient per week [Table 4].

The average number of patient per week in Dentistry Department in 2013 was 62/7.1mA–min/Wk, 2014 was 83/9.3mA–min/Wk, 2015 was 65/7.3mA–min/Wk and 2016 was 47/5.2mA–min/Wk, with 2013 having the highest number of patient per week [Table 4].

Discussion

Total effective dose received by radiographers in Radiology Department in 2013 was the highest (1.58 mSv) which represent 44.9% of the total dose received in that year; radiologist had a total dose of 1.39 mSv which was 39.5% of the total dose; followed by technicians who had a total dose of 0.39 mSv which was 11.1% of the total dose, while porters and health attendance /Nurse had 0.16 mSv corresponding to 4.5% of the total dose. The highest recorded dose was from Radiologists who were engaged with special examination while using the fluoroscopy unit.

In 2014, radiographers dose reduced by 72.2% against 2013 dose value and radiologist dose reduced by 83.5% in 2014 against 2013 dose value. This reduction was attributed to dose values that were below reportable dose (BRD) from the instadose™ readout of some personnel which was necessitated by awareness and compliance with simple radiation safety rules during exposure. The highest dose in 2014

Table 4. Average workload from 2013-2016 in Radiology and Dentistry Department

Radiology Department		
Year	Average number of patient per week	Workload (W) (mA-min/Wk)
2013	187	340
2014	159	290
2015	185	328
2016	160	242

Dentistry Department		
Year	Average number of patient per week	Workload (W) (mA-min/Wk)
2013	62	7.1
2014	83	9.3
2015	65	7.3
2016	47	5.2

was from the radiologist unit (0.12 mSv) which was 76% lower than the highest dose value in 2013. Although, the highest dose value was far below 20 mSv IAEA limit in a year [6, 7, 26, 27].

Radiographers dose record further reduced by 79.7% in 2015 against 2013 record and radiologist dose slightly increased from 16.6% to 17.3% from 2014 to 2015. The highest dose was still seen to be among the radiologist, while the least dose was from the health attendant/ nurse (0.04 mSv). In 2016, radiographer dose record was 80.4% lower than 2013 record with that of the radiologist being 85.6% lower than 2013 dose record. The highest (radiologist) and least dose (technician) were 0.2 mSv and 0.04 mSv, respectively. In general, radiologist effective doses were the highest. The next are radiographer who must times stay behind the lead screen or shield during radiographic, mammographic and fluoroscopic examinations. The dose received by the radiologist, radiographer and health attendance who work closely with the patient might largely be due to secondary radiation which are usually scatter radiation from patient or any other material in the X-ray room or leakage radiation from the tube head.

Annual mean effective dose in radiology department (using conventional, fluoroscopic and mammographic X-ray) varied in the range of 0.03-0.50 mSv over four years. This value was seen to be below a study conducted in 2010 using TLDs by Jabeen *et al.*[28] in Pakistan whose annual average effective dose in Diagnostic Radiology (DR) using conventional, fluoroscopic and mammographic X-ray machines from 2003-2007 ranged from 1.22-1.71mSv. Also this study's mean annual dose (0.17 mSv) in radiology department was lower compared to Nassef and Kinsara's study [29] whose annual mean dose was 0.66 mSv in diagnostic radiology using TLDs. Radiologist mean effective dose in this study was 0.23 mSv with range of 0.08-0.5 mSv; it was lower compared to Nassef and Kinsara's study [29] whose mean effective dose was 0.39 mSv with a range of 0.09-1.49 mSv.

Evaluation of dose value in Radiology Department between 2013 and 2014 show that difference existed in personnel dose (p=0.001), similar result was noticed between 2013 and 2015 in personnel dose (p=0.002), continuous trend was noticed between 2013 and 2016 likewise in personnel dose (p=0.002). There was no difference in personnel dose between 2014 and 2015 (p=0.171), also no significant difference was noticed between 2014 and 2016 (p=0.643) and between 2015 and 2016 in personnel dose (p=0.970).

The average number of patient per week and workload from 2013-2016 in Radiology Department were: 187/340, 187/290, 187/328 and 187/242mA-min per week; respectively with no significant correlation between personnel dose and workload (p=0.394) [Table 3]

Dental technicians in 2013 had a total dose of 0.53 mSv which accounted for 72.6% of the total dose received and the total dental surgeon dose was 0.2 mSv which translated to 27.4% of total dose received. Reduction of dose was noticed in 2014 for technicians (with total wearer of eight) which were 42.5% lower than those obtained in 2013 with total wearer of nine. It was noticed that two technicians dose value were below reportable dose throughout the year. Total dose in 2015 for technicians were 0.2 mSv with a dose reduction of 20% from 2014 (where technicians total dose was 0.25 mSv). There was reduction in the

number of technician and dental surgeon because their dose values were below reportable dose throughout the year. 2016 technician dose value was 5% lower than 2015. There was gradual reduction in the number technicians (Table 3), this was due to the fact that some personnel dosimeters were below reportable dose. Generally dose values in the dental department were below 20 mSv annual dose limit [6, 7, 26, 27].

A study conducted by Gray *et al.* [30] using TLD investigated the dose received by dental staff using Intra-oral X-ray unit. Measured mean monthly dose was 0.0078 mSv; this value was lower compared to our study which was 0.005 mSv/month with the DIS dosimeter.

Evaluation of dose value between 2013 and 2014 show that difference existed in personnel dose (p=0.021), deviation in result was noticed between 2013 and 2015 in personnel dose (p=0.274), between 2013 and 2016 personnel dose was different (p=0.038). There was no difference in personnel dose between 2014 and 2015 (p=0.627), also no significant difference was noticed between 2014 and 2016 (p=1.000) and between 2015 and 2016 in personnel dose (p=0.484).

The average number of patient per week and workload from 2013-2016 in Dentistry Department were: 62/7.1, 83/9.3, 65/7.3 and 47/5.2mA-min per week respectively with no significant correlation between personnel dose and workload (p=0.413) [Table 4].

The Limitations of the Study

Personnel not regularly wearing their DIS dosimeter was challenging in getting accurate dose record most especially among radiographers, radiologist and dental surgeons. Another limitation was “below reportable dose” which eventually count personnel dose as insignificant.

Conclusions

The first year of the DIS dosimeter had the highest dose in both departments but as the year progresses personnel dose gradually reduces due to radiation protection training that was implemented. Personnel dose report in radiology and dentistry department was generally below IAEA/ICRU annual dose limit of 20 mSv averaged over a period of 5 consecutive years.

Authorship declaration

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Contributions

ADO: involved in concept, design, literature search, data acquisition and analysis, statistical analysis and manuscript preparation, editing and reviewing, MOA: involved in design, literature search, data acquisition and analysis, statistical analysis and manuscript preparation, editing and reviewing, SOA: involved in literature search, data analysis, statistical analysis and manuscript preparation, UPA: involved in literature search, data analysis and statistical analysis, MIA: involved in data analysis and statistical analysis, MEE: involved in literature search and manuscript reviewing and CBM: involved in data acquisition and manuscript editing

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MicroRNA-33a levels do not correlate with the expression of its host gene SREBF2 and its isoforms in prostate cancer cell lines

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ABSTRACT

Objective. Prostate cancer is currently the most frequently diagnosed malignant neoplasm and the second leading cause of cancer related mortality in men over the age of 50 years in the developed countries. MicroRNA-33a (miR-33a), localized within the intron 16 of SREBF2, has been reported to have tumor suppressive properties in some cancers including prostate cancer, whereas its host gene, SREBF2, has been shown to be elevated in prostate cancer and to act as an oncogene. Due to the paradoxical expression of an oncogene and a tumor suppressor from a single genetic locus, there is a need for evaluation of miR-33a and SREBF2 expression status in prostate cancer cells to help understanding their roles in prostate carcinogenesis. ***Methods.*** In this study, we aimed at investigating the link between the expressions of miR-33a and its host gene SREBF2 and its isoforms in prostate cancer cell lines using quantitative real time PCR. We evaluated the relative expression levels with using $2^{-\Delta\Delta CT}$ method and tested the correlations of microRNA and gene expressions with Pearson's Correlation test using GraphPad Prism 6. ***Results.*** Our results demonstrated variable expression levels for SREBF2 mRNA and miR-33a expression levels in prostate cancer cell lines, with some decreased, some increased and some unchanged. Further analysis showed a strong correlation among expressions of SREBF2 isoforms though we could not find a significant association between levels of SREBF2 isoforms and miR-33a expression. ***Conclusion.*** This data suggest possible posttranscriptional regulation of miR-33a expression in prostate cancer.

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Keywords: Prostate cancer, miR-33a, SREBF2, correlation

Introduction

Prostate cancer (PCa) is the most commonly diagnosed non-skin cancer and the second leading cause of cancer deaths in males over the age of 50 years in the developed countries [1]. Emerging evidences suggest a high-cholesterol Western diet as an importantrisk factor for several solid tumors

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including PCa [2]. Although contradictory findings are present about the link between serum cholesterol levels and PCa risk, accumulating data supports a critical role for HDL, LDL and, total cholesterol in PCa development and progression [3-6]. In line with those studies, prostate tumor cells have been postulated to acquire castration-resistance via reactivating intrinsic androgen biosynthesis pathway, which might be through acquisition ofthe ability to synthesize androgens from its precursor, cholesterol [7].

The sterol regulatory element-binding protein (SREBP) transcription factors, SREBP1 and SREBF2, are among the crucial modulators of cholesterol/lipid homeostasis, and of those, SREBF2 upregulate genes associated with cholesterol synthesis (e.g. HMGCR) and cholesterol uptake (e.g. LDLR) [8]. In addition, microRNA-33a (miR-33a), localized within the intron 16 of the SREBF2 gene (Figure 1), which encodes SREBF2 protein, has been also reported to play important roles in cholesterol synthesis and uptake through targeting 3' untranslated regions of ABCA1, a cholesterol efflux protein, and several other mRNAs for proteins implicated in β-oxidation of fatty acids including CPT1A and HADHB [9].

Paradoxically, increase in SREBF2 but decrease in miR-33a level lead to increased cholesterol synthesis and uptake, although their expressions are controlled by the same promoter. Furthermore, SREBP2 has been reported to be increased in PCa and our recent findings imply tumor suppressive activities for miR-33a with decreased expression in PCa tissues [10]. Therefore, a comprehensive evaluation of this paradoxical expression pattern of miR-33a and SREBF2 in prostate cancer cells is necessary to help understanding their roles in prostate carcinogenesis.
In this study, we aimed at investigating the link between the expressions of miR-33a and its host gene SREBF2 and its isoforms in PCa cell lines.

Methods

Cell Culture

Immortalized non-tumorigenic prostate cell line PNT1a cells, LNCaP, DU145, 22RV1 and PC3 cells were grown using RPMI medium (GenDepot) containing 10% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin (Invitrogen). VCaP cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) supplemented with 10% FBS and 1% penicillin/streptomycin. All cell lines were cultured at 37°C in a humidified and 5% CO2 incubator. Cell lines were obtained from American Type Culture Collection and routinely authenticated by STR analysis at MD Anderson Cancer Center Characterized Cell Line Core Facility.

cDNA Synthesis and Quantitative Real-Time PCR

For microRNA first strand DNA (cDNA) synthesis, equal amounts of total RNA were reverse transcribed using microRNA specific primers (Applied Biosystems) and TaqMan MicroRNA reverse transcription Kit (Applied Biosystems) following the manufacturer's instructions. cDNA synthesis from mRNA was carried out with "amfiRivert cDNA Synthesis Platinum Master Mix" (GenDepot) following the manufacturer's protocol.

For microRNA expression analysis, microRNA specific probes (Applied Biosystems) and TaqMan Fast Advanced Master Mix (Applied Biosystems) were used. MicroRNA expression data were normalized to RNU43. For gene expression analysis, SYBR Green PCR Master Mix (Applied Biosystems) was used. Expression data were normalized to ь-actin. Primer sequences used for quantitative real time PCR (qRT-PCR) are provided in Supplementary Table1.

qRT-PCR was performed in a StepOnePlus™ real-time thermal cycler (Applied Biosystems) using standard parameters. Each experiment was performed

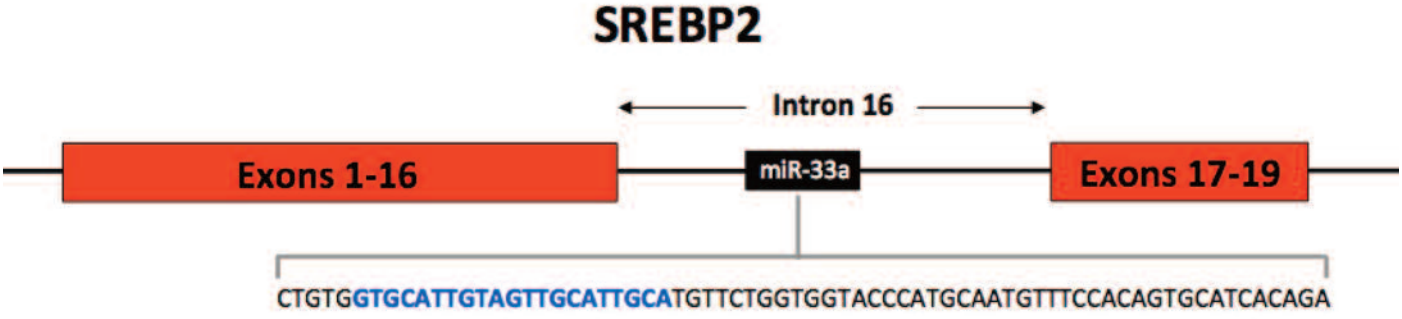

Figure 1. Schematic representation of the localization of miR-33a within 16th intron of SREBF2

in triplicates and the differences in expression levels were evaluated using $2^{-\Delta\Delta CT}$ method.

Statistical Analysis

Data were plotted as mean ± standard error and statistical significances were evaluatedusing Student's t test. Correlations of microRNA and gene expressions were analyzed with Pearson's Correlation testusing GraphPad Prism 6. A p value of 0.05 or below was accepted as significant.

Results

MiR-33a and SREBF2 have variable expression in PCa cell lines

To evaluate the correlation of miR-33a and SREBF2 expression, we initially measured their levels in PNT1a and PCa cell lines using qRT-PCR. MiR-33a expression was significantly reduced in LNCaP and VCaP cells and was significantly increased in 22RV1 and PC3 cells (Figure 2A). Its expression in DU145 cells was similar to that of PNT1a cells (Figure 2A). We also found variable expression levels for SREBF2 mRNA in PCa cell lines, with some decreased (PC3; Figure 2B), some increased (VCaP, DU145, and 22RV1; Figure 2B) and some unchanged (LNCaP; Figure 2B). However, there was no correlation of SREBF2 and miR-33a levels in the same cancer cell lines (Table 1; p >.1, Pearson).

Expression of MiR-33a and SREBF2 isoforms do not correlate in PCa cell lines

We then examined the expression levels of SREBF2 isoforms (See Supplementary Table 1), which include intron 16 in their premature unspliced forms, in PCa cells lines to look for a specific isoform,

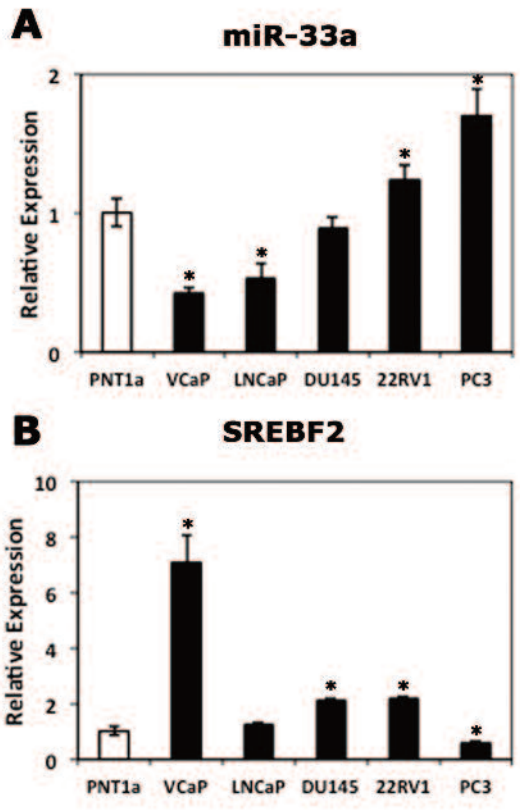

Figure 2. Relative expression of (A) miR-33a and (B) SREBF2 in PCa cells. Mean +/- SEM is shown.*$p < 0.05$

whose expression might be correlated with miR-33a expression. These isoforms represented similar expression profiles with total SREBF2 level (compare Figure 2B and Figure 3) and correlation analysis demonstrated that the levels of SREBF2 isoforms strongly related with each other in the same cancer cell lines, although they lack a significant correlation with miR-33a (Table 1).

Furthermore, we searched for the retained introns that include intron 16 and analyzed the expression level of a retained intron in PCa cell lines (See Supplementary Table 1). We designed a primer pair

Table 1. Correlation of SREBF2 mRNA and miR-33a levels

	SREBF2	SREBF2 001	SREBF2 002	SREBF2 005	SREBF2 001+201	Retained Intron
miR-33a	R = -0.616 *p* = 0.192	R = -0.662 *p* = 0.151	R = -0.450 *p* = 0.369	R = -0.671 *p* = 0.144	R = -0.617 *p* = 0.191	R = -0.648 *p* = 0.163
SREBF2		**R = -0.970** ***p* = 0.001**	**R = -0.933** ***p* = 0.006**	**R = -0.957** ***p* = 0.002**	**R = -0.993** ***p* = 0.0001**	**R = -0.959** ***p* = 0.002**
SREBF2 001			**R = -0.950** ***p* = 0.003**	**R = -0.997** ***p* = 0.0001**	**R = -0.993** ***p* = 0.0001**	**R = -0.959** ***p* = 0.002**
SREBF2 002				**R = -0.935** ***p* = 0.006**	**R = -0.949,** ***p* = 0.003**	**R = -0.945** ***p* = 0.004**
SREBF2 005					**R = -0.980** ***p* = 0.0005**	**R = -0.999** ***p* = 0.0001**
SREBF2 001+201						**R = -0.982** ***p* = 0.0005**

targeting intron 1 as control to exclude the possibility of amplifying genomic DNA. qRT-PCR results demonstrated no correlation of the retained introns expression to miR-33a levels (Figure 4).

Our overall results demonstrated a strong correlation among expression of SREBF2 isoforms althoughthere was nolink between expressions of SREBF2 isoforms and miR-33a level (Figure 5), which suggested possible posttranscriptional regulation of miR-33a expression in PCa.

Discussion

Numerous studies suggest a critical role for cholesterol in PCa development and progression in recent years [3-6]. In advanced prostate tumors, castration-resistance of tumor cells might occur via reactivation of intrinsic androgen biosynthesis pathways, where cholesterol might serve as an important precursor for synthesis of androgens [7].

There is strong evidence that in normal tissues,

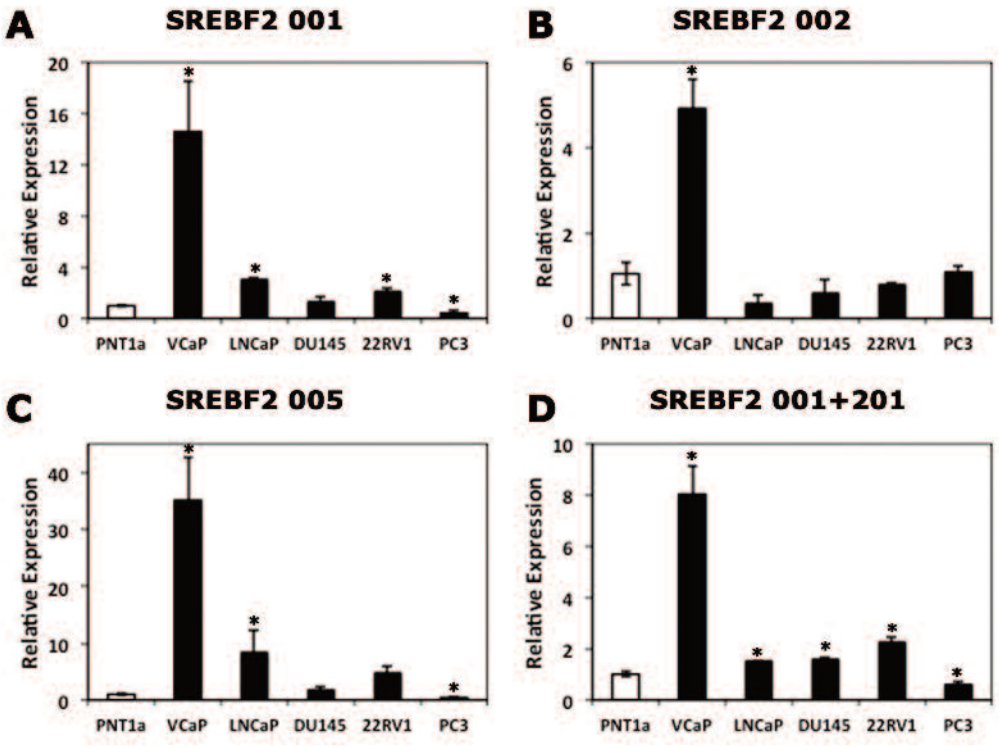

Figure 3. Relative expression of (A) SREBF2 001, (B) SREBF2 002, (C) SREBF2 005, and (D) SREBF2 001+201 in PCa cells. Mean +/- SEM is shown. *$p < 0.05$

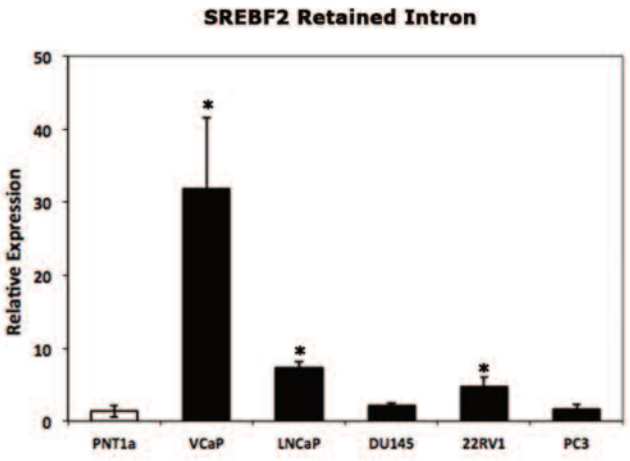

Figure 4. Relative expression of SREBF2 retained intron in PCa cells. Mean +/- SEM is shown. *$p < 0.05$

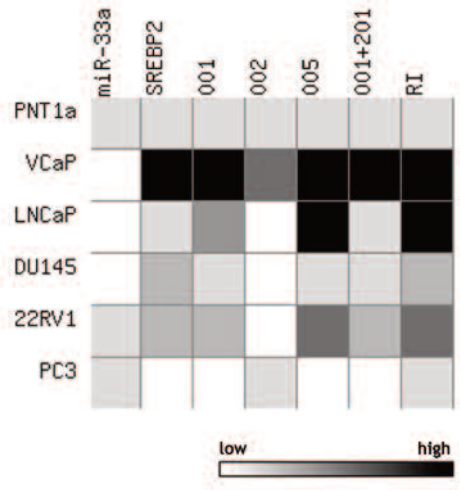

Figure 5. Heat-map representation of SREBF2 isoform and miR-33a relative expression levels in PCa cell lines. Mean +/- SEM is shown. *$p < 0.05$

miR-33a levels are elevated in parallel to increased SREBF2 transcription, leading to collaborative regulation of cholesterol and other lipid levels by SREBF2 and miR-33a [11]. In contrast to this finding, miR-33a, localized within the intron 16 of SREBF2, has been reported to have tumor suppressive properties in some cancers including PCa [10, 12-18], whereas its host gene, SREBF2, has recently been shown to be increased in PCa and to act as an oncogene [19]. Due to these paradoxical findings, there is a need for evaluation of miR-33a and SREBF2 expression status in PCa cells to help understanding their roles in prostate carcinogenesis.

Therefore, in this study, we investigated the association between the levels of miR-33a and its host gene SREBF2 and its isoforms in PCa cell lines and found that there is no correlation of SREBF2 isoform mRNA levels with its intronic microRNA miR-33a in PCa unlike the correlation seen in normal tissues.

In normal tissues, SREBF2 increase cholesterol by increasing transcription of multiple genes that increase levels of cholesterol. Elevated levels of SREBF2 in cancer tissues more profoundly induce upregulation of those genes associated with cholesterol synthesis and cholesterol uptake. Increased synthesis and uptake of fatty acids can pave the way for reactivation of intrinsic androgen biosynthesis pathways but also provide an energy source for PCa, which are known to have low glucose uptake. Besides, apart from lipid biogenesis, SREBP-2 was found to induce c-Myc expression via directly interacting with c-Myc promoter region to drive stemness and metastasis [19]. In addition, downregulation of miR-33a allows both upregulation of oncogenic genes such as PIM1 [10] and promotes β-oxidation of fatty acids through overexpression of genes like HADHB and CPT1A [9]. Such increased β-oxidation might contribute to providing of energy to PCa cells. Another potential association of miR-33a to cholesterol metabolism is its targets that are involved in cholesterol transport such as ABCA1, ABCG1, and NPC1 [9]. Several studies showed that upregulation of miR-33a in vitro profoundly suppressed cholesterol export in various cell culture models [20-23]. Further in vivo studies also demonstrated significant elevation in serum HDL cholesterol in miR-33a -/- mice [21]. However, it is worth mentioning that ABCA1, a cholesterol efflux protein, which is targeted by miR-33a, is significantly methylated in PCa [24], which would abolish the potential deleterious effects of elevated cholesterol efflux secondary to reduced miR-33a.

Furthermore, interestingly in 2 of the androgen receptor positive cell lines tested, LNCaP and VCaP cells, miR-33a expressions were lower compared to that of PNT1a. On the other hand, its expression was either unchanged or elevated in androgen receptor negative DU-145 and PC3 cells, implying a possible androgen receptor related mechanism for differential expression of miR-33a in PCa cells.

The Limitations of the Study

Our study focuses on the cell lines for the evaluation of miR-33a and SREPF2 isoforms' expression. Lack of the correlation of miR-33a and SREPF2 isoforms' expression in tumor and normal prostate samples obtained from PCa patients is one of the important limitations of our study. In addition, SREBF2 expression in protein was not evaluated in regards to its correlation with miR-33a expression.

Conclusions

The potential expression of an oncogene and a tumor suppressor from a single genetic locus creates a paradox in PCa. In this study, we show that miR-33a expression is not correlated with SREBF2 mRNA levels, implying post-transcriptional mechanisms of control of miR-33a levels in PCa, leading to decreased miR33a levels. We demonstrated a strong correlation among expressions of SREBF2 isoforms though we could not find a significant correlation between expressions of SREBF2 isoforms and miR-33a expression, which suggested possible post-transcriptional regulation of miR-33a expression in PCa. Further studies should be carried out to better understand the possible mechanisms of differential expression of miR-33a and SREPF2 isoforms in PCa cells although transcribed from a single locus. Also, further in vivo research is needed to clarify the roles of miR-33a and SREBF2 in PCa tumorigenesis process.

Authorship declaration

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Supplementary Table 1. QRT-PCR primer sequences and PIM1 3'UTR cloning and mutagenesis primer sequences

Primer	Ensembl Transcript ID	Sequence
Beta-actin-F		5'-GCCTCGCCTTTGCCGATC-3'
Beta-actin-R		5'-CCCACGATGGAGGGGAAG-3'
SREBF2-F		5'-CAGCCTCAAGTCCAAAGCCT-3'
SREBF2-R		5'-TGTCTTGATGATCTGAGGCTGG-3'
SREBF2-001-F	ENST00000361204	5'-CTCGCCAGAGGAGATTTTGC-3'
SREBF2-001-R		5'-TGGAAGACTTTCTTGAGCAGC-3'
SREBF2-002-F	ENST00000424354,	5'-TGTGCGCTCTCATTTTACCA-3'
SREBF2-002-R		5'-CGCAGACATGAATCTCCAAA-3'
SREBF2-005-F	ENST00000435061	5'-GTCCAGGGCTTTCTTGTCAC-3'
SREBF2-005-R		5'-CAGGCTGTGTTCCAGCAG-3'
SREBF2-001 + 201-F	ENST00000361204 + ENST00000612482	5'-TGGAAGTGACAGAGAGCCC-3'
SREBF2-001 + 201-R		5'-GTTGAGGGCAGGGTCAGAG-3'
Retained Intron-F	ENST00000490262	5'-GGCACACAAACAGAGCTGAA-3'
Retained Intron-R		5'-CCTTCAGTCAGGGCAGTCTC-3'
Control Intron-F		5'-GGCGGTCCTCAACCCTTC-3'
Control Intron-R		5'-AGAGCGGACCACGGAAAC-3'

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The effect of obesity on functional capacity, anxiety and daily life activities in patients with coronary artery disease and phase II cardiac rehabilitation

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ABSTRACT

Objective. The aim of our study was to investigate the effect of cardiac rehabilitation (CR) program on obese and non-obese patients with coronary artery disease. ***Methods.*** The records of 60 coronary artery disease patients aged between 18-75 years, who were included in CR were evaluated. Of the study subjects, 20 had undergone coronary artery bypass grafting (CABG) and 40 had undergone percutaneous coronary intervention (PCI). The patients were divided into two groups by body mass index (BMI), with Group I being non-obese (BMI < 30 kg/m2) and Group 2 being obese (BMI ≥ 30 kg/m2). The effect of 30 session CR on the two groups were evaluated with 6-min walk test (6MWT), Short Form-36 (SF-36) and Beck Anxiety Inventory (BAI). ***Results.*** Baseline characteristics of the group 1 (39 patients) and group 2 (21 patients) were similar. Statistically significant improvement was detected in group 1 and group 2 patients by CR program in 6MWT, BAI and SF-36 parameters ($p < 0.05$). 6MWT, BAI and SF-36 parameters changes by cardiac rehabilitation were compared between the two groups. According to the comparison 6MWT (group 1; 60 (20-183) vs group 2; 34 (15-180), $p = 0.012$) and MET (group 1; 1.44 ± 0.56 vs group 2; 1.09 ± 0.41, $p = 0.015$ changes were significantly higher in group 1 than in group 2. However, the changes of BAI and SF-36 parameters were similar in two groups ($p > 0.05$). ***Conclusion.*** CR was found to be effective and safe in terms of functional capacity, daily life activities and anxiety in both obese and non-obese patients. Functional capacity gain in the obese group was less than non-obese patients.

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Keywords: cardiac rehabilitation, obesity, quality of life, functional capacity, anxiety

Introduction

Cardiovascular diseases are associated with high morbidity and mortality risk despite the recent innovations in diagnosis and treatment. The prevalence of coronary heart diseases in Turkey has

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increased 1.4-2.2 times between 1990-2006, and there is an increase of 5% per year in people over the age of 60 [1]. In patients with coronary artery disease (CAD), functional capacity is a strong predictor of mortality and morbidity. Daily activities and exercise capacities of these patients are often restricted because of their cardiopulmonary and musculoskeletal limitations. Many studies have shown that risk factors for active patients are reduced relative to patients who are sedentary for developing CAD [2-5].

The World Health Organization's description of cardiac rehabilitation (CR) can be summarized as follows: "ensuring that cardiac patients regain their pre-disease health physically, mentally and socially as much as possible" [6]. The main goal of CR is to increase the daily activity of the cardiac patient, change the natural course of the illness and improve their quality of life. CR not only increases the functionality but also reminds the patient and their family to make lifestyle changes [7, 8]. Effective CR is an effective method for protection from cardiovascular morbidity and mortality [9].

Prevalence of obesity worldwide has doubled in recent years due to changes in diet and daily life activities [10, 11]. Obesity is known to have negative effects on the increase in mortality and morbidity in cardiovascular diseases [12-14]. Increasing number of obese patients increases the number of CADs [15, 16]. Due to negative effects of obesity on cardiac wellbeing, it may affect the results of CR program [14, 17-20].

The aim of our study was to investigate the effect of CR on obese and non-obese patients with CAD that were included in CR program and to compare their results.

Methods

In our study, the records of 60 coronary artery patients aged between 18-75 years, who were included in CR program of Physical Medicine and Rehabilitation clinic. The approval of the ethics committee and informed consent from the patients were obtained. Of the study subjects, 20 had undergone coronary artery bypass grafting (CABG) and 40 had undergone percutaneous coronary intervention (PCI). Patients with congestive heart failure, malignancy diagnosis, chronic obstructive pulmonary disease (COPD), progressive worsening of rest or exercise tolerance over the last 3-5 days, marked ischemia at low workload (< 2 MET or ~ 50 Watt), uncontrolled diabetes, acute systemic disease or fever, newly diagnosed embolism, thrombophlebitis, active pericarditis or myocarditis, moderate or severe aortic stenosis, regurgitant valvular heart disease requiring surgery, myocardial infarction within the last 3 weeks, patients with new-onset atrial fibrillation and bypass surgery were excluded from the study. The patients were taken to the rehabilitation program 4 weeks after the intervention. All patients underwent treadmill exercise stress test (Schiller CS-200 Excellence, 2006, Swedish) using Bruce protocol in the presence of a cardiologist in the beginning and end of the CR. The exercise stress test allowed determination of metabolic equivalent (MET) hemodynamic response markers and evaluation of functional capacity.

Data of the patient who completed 30 sessions of phase II CR program 4 days a week were evaluated. CR included 5 minutes of warm-up exercises, 10 minutes of joint range of motion exercises, 10 minutes of stretching exercises, 30 minutes of cycling with aerobic exercise (Custo-med 2014, Germany) and 5 minutes of cooling exercises. After 2 weeks, the patients were continued on the 60-minute rehabilitation, to which resistive exercises were added, and the intensity of exercise was increased by 15% once every 2 weeks. During exercise, the patients' pulse oximeter, arterial blood pressure, heart rate and rhythm, oxygen saturation were monitored. All patients were included in the exercise program based on their maximum heart rate achieved. They were made to exercise with 60-85% of their maximum heart rate achieved. We did not observed any complication. The patients were divided into two groups by body mass index (BMI), with Group I being non-obese (BMI < 30 kg/m2) and Group 2 being obese (BMI ≥ 30 kg/m2).

In our study, using 6-min walk test (6MWT), SF-36 and Beck Anxiety Inventory (BAI), pre-treatment and post-30-session data of CAD patients who completed cardiac rehabilitation program were evaluated.

Evaluation Parameters

–The 6-minute walk test (6MWT):

The 6MWT is a test to measure exercise tolerance in people with lung or cardiac disease. The longer the walking distance, the better the patient's functional status [21].

–Short Form (SF-36):

The SF-36 is a recognized scale often used to evaluate quality of life. It is not specific to any disease group. It consists of 36 items and 8 subscales related to physical health (physical functioning, physical role, pain, general health) and mental health (energy, social function, role limitations due to emotional problems, mental health). Each subscale is scored in the range of 0 to 100. A high score indicates good health status. It was adapted for Turkish society and found to be valid and reliable in chronic low back pain. Kocyiğit et al. performed reliability and validity study of the scale in Turkey in 1999 [22].

–Beck Anxiety Inventory (BAI):

The inventory measures the frequency of anxiety symptoms. It is a 21-item self-report inventory that is scored in the range of 0-3. The person is asked to tick one of the following options "Not At All", "Mildly", "Moderately" and "Severely". The scoring range is 0-63. The higher the total score, the higher the level of anxiety of the person. A total score of 8-15 is interpreted as "Mild" level of anxiety; 16-25 as "Moderate", and 26-63 as "Severe". The BAI was created by Beck et al. (1988) and its validity and reliability study in Turkey was performed by Ulusoy et al. (1998) [23, 24].

Statistical Analysis

An IBM SPSS 22.0 Statistics software was used to analyze data obtained through the study. In assessment of the data, chi-square (χ^2) test was used to compare categorical data andFisher's exact test was used to assess differences in the comparison of gender. Descriptive statistical methods (frequency, percentage, mean, standard deviation, median, min–max) were used to assess the study data. Normal distribution of the data was tested using Shapiro-Wilk test. Independent Samples t test (t test for independent groups) was used in between-groups comparisons while variables were found normally distributed. When a normal distribution was not foundMann Whitney U test for comparisons between groups were used. Intra group repeated measurements (before and after CR) were analyzed with the Wilcoxon signed rank test. Values with a probability of (p) **a**< 0.05 was accepted as significant.

Results

Mean age of 60 patients included in the study was 52.65 ± 10.81, and the patients were divided into two groups by BMI. Thirty-nine patients had a BMI of 30 kg/m2 and less (Group 1) and 21 patients had a BMI of 30 kg/m2 and more (Group 2). Only 10 patients in Group 1 had a BMI less than 25 kg/m2. 18 (30%) patients had hypertension, 15 (25%) had diabetes, 11 (18.3%) had hyperlipidemia and 14 (23.3%) were

Table 1. Baseline demographic characteristics of the groups

Characteristic	Group 1 (n=39)	Group 2 (n=21)	*p* value
Age	50.56 ± 10.42	56.19 ± 10.59	0.076
Gender			0.528
Female	6 (15.4%)	2 (9.5%)	
Male	33 (84.6%)	19 (90.5%)	
Cigarette			0.485
Current smoker	8 (20.5%)	6 (28.6%)	
No-oldsmoker	31 (79.5%)	15 (71.4%)	
Diagnosis			0.574
CAD	27 (69.2%)	13 (61.9%)	
CABG	12 (30.8%)	8 (38.1%)	
Disease duration (year)	3.90 (1-10)	5.57 (1-15)	0.122
Additional disease			0.234
HT	10 (25.6%)	8 (38.1%)	
DM	8 (20.6%)	7 (33.3%)	
Hiperlipidemia	7 (17.9%)	4 (19%)	
BMI (kg/m^2)	26.29 ± 2.60	31.62 ± 1.05	**< 0.001**

The values were presented as mean ± SD (standard deviation) or number (percent. BMI = body mass index, HT = hypertension, DM = diabetes mellitus, CABG = coronary artery bypass grafting, CAD = coronary artery disease, MET = metabolic equivalent, $p < 0.05$ statistically significant

Table 2. The baseline 6MWT, BAI and SF-36 parameters of the two groups

Pre-treatment	Group 1 (n = 39)	Group 2 (n = 21)	*p* value
6 minwalk test (m)	428 (340-545)	405 (330-585)	0.828
MET	8.56 ± 1.14	8.53 ± 1.02	0.907
BECK anxiety inventory	12 (1-33)	10 (3-34)	0.624
SF-36 physical functıon	52.82 ± 25.72	54.52 ± 21.14	0.864
SF-36 physical role	12.50 (0-50)	12.50 (0-50)	0.306
SF-36 emotional role	16.66 (0-85)	16.66 (0-83.33)	0.961
SF-36 energy-vitality	53.48 ± 20.38	52,92 ± 23,46	0.938
SF-36 mental health	62.50 (16-96)	68 (16-92)	0.544
SF-36 social function	62.50 (12.50-100)	75 (25-100)	0.382
SF-36 pain	67.50 (20-100)	57.50 (20-100)	0.803
SF-36 general health	51.28 ± 19.62	48.57 ± 15.74	0.619

The values were presented as mean ± SD (standard deviation) or median (maximum-minimum). 6MWT = 6 min walk test, BAI = Beck anxiety inventory, CR = cardiac rehabilitation, MET = metabolic equivalent, SF-36 = short form 36, $p < 0.05$ statistically significant

smokers. Comparative values of both groups by demographic characteristics and by initial assessment parameters are shown in Table 1 and 2, respectively. No significant difference was determined between the groups.

When Group 1 patients were assessed at the end of 30 sessions of CR program, a statistically significant improvement was detected in 6MWT, BAI, MET, SF-36 physical functioning, physical role, mental health, social functioning and pain subparameters (Table 3).

When Group 2 patients were assessed at the end of 30 sessions of CR program, a statistically significant improvement was detected in 6MWT, BAI, MET, SF-36 physical functioning, physical role and pain subparameters (Table 4).

When the groups were compared by difference scores, a statistically significant difference was determined in 6MWT and MET value in favor of group 1; no significant difference was identified in other assessment parameters (Table 5).

Discussion

We aimed to investigate whether obesity changes the effect of phase ll CR on functional capacity, daily life activities and anxiety or not. According to our results, CR had favorable effects on functional capacity, anxiety and daily life activities of all patients, however, functional gain in non-obese group was

Table 3. The effect of cardiac rehabilitation program on 6MWT, BAI and SF-36 parameters in group 1

Group 1 (n = 39)	Before CR	After CR	*p* value
6 min walk test (m)	428 (340-545)	517 (360-611)	**< 0.001**
MET	8.56 ± 1.14	10.01 ± 1.16	**< 0.001**
Beck anxiety inventory	12 (1-33)	7 (1-30)	**< 0.001**
SF-36 physical function	52.82 ± 25.72	61.98 ± 23.50	**0.002**
SF-36 physical role	12.50 (0-50)	25 (0-50)	**0.002**
SF-36 emotional role	16.66 (0-85)	33.33 (0-50)	0.151
SF-36 energy-vitality	53.48 ± 20.38	58.84 ± 17.70	0.100
SF-36 mental health	62.50 (16-96)	76 (12-100)	**0.012**
SF-36 social function	62.50 (12.50-100)	87.50 (25-100)	**0.001**
SF-36 pain	67.50 (20-100)	87.50 (32-100)	**< 0.001**
SF-36 general health	51.28 ± 19.62	53.33 ± 23.09	0.304

The values were presented as mean ± SD (standard deviation) or median (maximum-minimum). 6MWT = 6 min walk test, BAI = Beck anxiety inventory, CR = cardiac rehabilitation, MET = metabolic equivalent, SF-36 = short form 36, $p < 0.05$ statistically significant

Table 4. The effect of cardiac rehabilitation program on 6MWT, BAI and SF-36 parametersin group 2

Group 2 (n = 21)	Before CR	After CR	*p* value
6 min walk test (m)	405 (330-585)	446 (364-600)	**< 0.001**
MET	8.53 ± 1.02	9.62 ± 1.13	**< 0.001**
Beck anxiety inventory	10 (3-34)	4 (2-21)	**0.003**
SF-36 physical function	50 (25-85)	70 (37.50-85)	**0.001**
SF-36 physical role	12.50 (0-50)	25 (0-50)	**0.034**
SF-36 emotional role	16.66 (0-83.33)	33.33 (0-50)	0.727
SF-36 energy-vitality	52.92 ± 23.46	56.90 ± 19.58	0.302
SF-36 mental health	63.47 ± 18.45	65.71 ± 18.48	0.603
SF-36 social function	75 (25-100)	75 (37.50-100)	0.297
SF-36 pain	57.50 (20-100)	77.50 (45-100)	**0.001**
SF-36 general health	48.57 ± 15.74	52.61 ± 16.47	0.225

The values were presented as mean ± SD (standard deviation) or median (maximum-minimum). 6MWT = 6 min walk test, BAI = Beck anxiety inventory, CR = cardiac rehabilitation, MET = metabolic equivalent, SF-36 = short form 36, $p < 0.05$ statistically significant

more prominent.

Being overweight and obesity have been reported to have many negative effects on hemodynamicand cardiovascular status and function. According to World Health Organization, a BMI ≥30 kg/m^2 indicates obesity, 25-29.99 kg/m^2 the overweight range, 18.50-24.99 the normal range, and <18.50 underweight range [11, 25]. 80% of cardiac rehabilitation patients consists of obese and overweight patients [26, 27]. In the case of our patients, in line with the literature, only 10 (16.6%) patients were in the normal range (BMI <25 kg/m2), with 29 (48.33%) patients being overweight and 21 (35%) patients being obese. No extremely obese patient was observed.

In a previous study, initial and post-CR work capacities of obese patients were found to be less [27]. Similarly, Seres et al. [28] identified a decrease in exercise performance in the case of obesity and observed lesser functional gain in each session of CR in the case of obesity. Contrary to this study, Lavie and Milan [18] reported equal level of post-CR improvement in obese and non-obese patients groups. In another study on the assessment of the effect of CRP on obese and non-obese patients who had acute MI, a significant increase was observed in MET values of both groups, and no superiority of the results of one group over the other could be detected [20]. Similarly, we found an equal level of functional capacity in obese and non-obese patient groups in initial assessment

Table 5. Comparison of 6MWT, BAI and SF-36 parameters changes with cardiac rehabilitation between the two groups.

Variation	Group 1	Group 2	*p* value
6 min walk test (m)	60 (20-183)	34 (15-180)	**0.012**
MET	1.44 ± 0.56	1.09 ± 0.41	**0.015**
Beck anxiety inventory	-4 (-27-1)	-5 (-31-3)	0.674
SF-36 physical function	12.50 (-45-40)	10 (0-40)	0.814
SF-36 physical role	11.32 ± 21.02	8.73 ± 17.57	0.723
SF-36 emotional role	0.0 (-51,67-50)	0.0 (-66.67-33.33)	0.714
SF-36 energy-vitality	7.5 (-38-49)	5 (-35-32.0)	0.950
SF-36 mental health	8 (-60-47)	4 (-60-48)	0.079
SF-36 social function	12.50 (-17.50-67.50)	0 (-25-50)	0.143
SF-36 pain	5 (-30-30)	12.50 (-22.50-47.50)	0.937
SF-36 general health	2.05 ± 12.28	4.04 ± 14.80	0.314

The values were presented as mean ± SD (standard deviation) or median (maximum-minimum). 6MWT = 6 min walk test, BAI = Beck anxiety inventory, MET = metabolic equivalent, SF-36 = short form 36, $p < 0.05$ statistically significant

parameters. This may be ascribed to the fact that BMIs of the patients in non-obese group fell within the overweight range (25-30 kg/m2). Post-CR exercise tolerance and functional capacity increased in both obese and non-obese patients, however, this increase was more significant in the non-obese group.

In a study evaluating post-CR exercise capacity and quality of life in patients with left ventricular dysfunction, the MET values increased from 8.00 ± 2.56 to 10.08 ± 3.00 MET, with a significant improvement in the SF-36 physical and psychological component of the patients [29]. In our study, we observed a significant difference in the MET values and quality of life scales of both groups of patients, however, the increase in MET values was more prominent in the non-obese group.

6MWT is a test frequently used to evaluate functional capacity, follow-up and prognosis in CAD patients. 6MWT was found to have a strong level of evidence in a meta-analysis performed to evaluate the efficiency of CR [21]. In a prospective, longitudinal observational study, 6MWT was again reported to be a repeatable, effective and reliable method to evaluate the results of CR [30].

Obesity is associated with reduced exercise performance, which could ultimately result in smaller gains in functional work capacity during CR program. The lower exercise capacity gain with CR in obese patients might be related with these patients limited functional capacity.

In a study evaluating post-CR quality of life of patients using SF-36, a significant improvement was observed in physical functioning, role limitations due to physical problems, emotional role, energy, social function and pain, with no difference in general health perception and mental health [31]. In another study using SF-36, a significant increase was identified in post-CR physical role, physical functioning, energy, pain and emotional, social, mental wellbeing [32]. In line with the said study, we found a significant improvement in physical role, physical functioning, mental health, social functioning and pain, which are SF-36 parameters, in the non-obese group. There was no significant difference in emotional role, energy and general perception of health. In the obese group, a significant difference was observed only in physical role, physical functioning and pain, whereas there was no significant difference in other parameters.

In a study evaluating anxiety using BAI in phase II CR, mild (BAI > 8) level of anxiety was reported in 41% of the patients and BAI was found to be a valid method in CR [33]. In an exercise-based rehabilitation program evaluating post-MI depression and anxiety levels, a decrease was detected in depression and anxiety levels of females, with no difference in males [34]. In our study, 39 patients had a BAI > 8 and 8 female patients all had a BAI > 8. In post-CRP period, a significant improvement was observed in anxiety levels of all patients.

In a study on the assessment of 30 sessions of CR on quality of life, functional capacity in CAD patients who underwent CABG, a significant improvement was observed in exercise capacity, functional capacity, quality of life scales. Only CAD patients had significant improvement in depression level after CR [35]. Similar to that study, we observed a significant improvement in SF-36 quality of life scale and functional activity in our patients. We also identified a significant improvement in anxiety scores.

Previous studies reported cardiac arrest, myocardial infarction, sudden cardiac death as complications during CR [36, 37]. As a result of our 30-session cardiac rehabilitation program, no complication was observed.

The Limitations of the Study

The main limitation of this study was relatively small number of patients. The study patients consisted of two different groups, CABG and PCI patients. So, the inclusion of a larger and more consolide group of patients would provide a more comprehensive picture. There was not follow up, medium and long-term follow-up results may be more descriptive about clinic effects.

Conclusions

In this study, CR was found to be effective and safe in terms of functional capacity, daily life activities and anxiety in both obese and non-obese patient groups. Functional gain in the obese group was less than non-obese group. Further studies based on larger populations are needed.

Authorship declaration

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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In-vitro transfection potential of fluorinated G5 PAMAM dendrimers for miRNA delivery to MRC-5 cells

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ABSTRACT

Objectives. MicroRNAs (miRNAs) are involved in the regulation of most biological processes and also contribute to many types of disease. Fibroblast cells, such as MRC-5, are often used in biological researches utilizing cell transfection methods due to their difficult to transfect nature. Cells can be genetically engineered by using viral and non-viral methods. Poly(amidoamine) (PAMAM) dendrimers are very promising alternative as a delivery vehicle due to their well-defined characteristics. In this study, in vitro transfection potential of cystamine core generation five (G5) PAMAM dendrimers fluorinated with 2,3,4,5,6-pentafluorobenzoic acid (PFB) and pentafluoropropionic acid (PFP) for miRNA delivery to MRC-5 cells was examined. ***Methods.*** Spectroscopic techniques were used in the characterization of the prepared dendrimers. miRNA binding and condensation capability of dendrimers was examined by gel retardation assay. Characterization of dendriplexes was made by zeta potential, particle size measurements and transmission electron microscopy. Transfection efficiencies of the dendriplexes were determined by flow cytometry and intracytoplasmic distribution of the dendriplexes was shown by laser scanning confocal microscopy. Also, quantitative structure-activity relationship and molecular docking calculations were used to be able to discuss transfection efficiencies of the dendriplexes into the cell. ***Results.*** While high level of viability on MRC-5 cells was observed for dendriplexes prepared with PFB and PFP, transfection efficiency with PFP was higher than PFB. Transfection efficiency difference between these two compounds was attributed to their molecular structures. ***Conclusions.*** Obtained results hold promise for the usage of these compounds as a transfection reagent at MRC-5 cells. Further studies are needed to support these findings.

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Keywords: Cystamine-G5-PAMAM, pentafluorobenzoic acid, pentafluoropropionic acid, miRNA, MRC-5

Introduction

MicroRNAs (miRNAs) are one of the RNA-interference based therapeutics vehicles that will be able to be effective during the genetic engineering process if they successfully transfected into the cell [1, 2]. However, direct administration of miRNAs in vitro or in vivo is not efficient due to their low cellular

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internalization and enzymatic degradation [3]. Researches towards to the development of efficient nucleic acid carriers, transfection reagents, are being carried out in order to bypass these drawbacks.

Poly(amidoamine) (PAMAM) dendrimers are cationic, hyper-branched, highly symmetric, nano-sized, three-dimensional macromolecules with well-defined structure [4]. Their chemical homogeneity and presence of multiple surface groups suitable for the binding of different target molecules makes them potential candidates for medical or biological applications [5, 6]. Polycationic dendrimers such as PAMAM possess primary amine groups at the surface, which participate in the DNA/small interfering RNA (siRNA) binding process and increase their cellular uptake by transforming the entire complex into nanoscale polyplexes. Also, bioreducible PAMAMs such as prepared by N,N'-dimethylcystamine (DMC) or N,N'-cystaminebisacrylamide (CBA) can provide selective intracellular release of nucleic acids [6-10]. However, these highly efficient delivery systems have been less explored for miRNA delivery [11, 12].

Despite mentioned characteristics of the dendrimers, their transfection efficiencies remain relatively low when compared to the viral vectors. For this reason, studies towards to increase transfection efficiency and biocompatibility of these polymers are being conducted. Fluorination is one of the methods used for this purpose. Fluorination improves the affinity of dendrimers to the cell membrane and also makes them able to cross the lipid bilayer of the cell membrane, as well as the endosome/lysosome membrane [13, 14].

In this study, in vitro transfection potential of fluorinated generation five (G5) PAMAM dendrimers for miRNA delivery to MRC-5 cells has been investigated. Cystamine core G5 PAMAM dendrimers were modified with 2,3,4,5,6-pentafluorobenzoic acid (PFB) and pentafluoropropionic acid (PFP) and these dendrimers were used for miRNA delivery to MRC-5 cells. In addition, the effect of structural differences of fluoro compounds over transfection efficiency was discussed by molecular docking calculations.

Methods

Materials

2-mercaptoethanol, cystamine dihydrochloride, ethylenediamine, ethyl acrylate, triethylamine, 2,3,4,5,6-pentafluorobenzoic acid, pentafluoropropionic acid and methanol were purchased from Sigma-Aldrich (Germany). The chemicals were used as received without further purification.

Synthesis and characterization of dendrimers

G5 PAMAM: Divergent method described previously was slightly modified for the synthesis of cystamine-core amine-terminated G5 PAMAM dendrimer [15]. During the synthesis, azeotropic evaporation, high vacuum or ultrafiltration with MWCO of 3000 and 10000 Da methods were used for product purifications. The products were characterized by 13C-NMR and FT-IR at the end of each step of the synthesis and also 1H-NMR, ESI-MS and MALDI-TOF-MS techniques were used when required.

Fluorinated G5 PAMAM: Fluorinated G5 PAMAM dendrimers were synthesized by adding methanolic solutions of 32 equivalent-grams PFB and PFP into methanolic solution of 1 equivalent-gram G5 PAMAM dendrimer and the mixtures stirred at room temperature for 48 h. The products were lyophilized to obtain fluorinated dendrimers as pale-yellow gels. The products were characterized by ^{19}F-NMR.

Preparation and characterization of the fluorinated dendrimer/miRNA dendriplexes

Fluorinated dendrimers and miRNA (miRIDIAN microRNA Mimic Negative Control #1, Dharmacon) were mixed in DNase/RNase free water at different w/w ratios; PFB: 3.75:1, 7.5:1, 15:1, 20:1, 40:1, 80:1, PFP:0.7:1, 1.4:1, 3.5:1, 7:1, 14:1, 21:1. The dendriplexes were incubated for 30-60 min before characterization.

miRNA binding and condensation capability of the fluorinated dendrimers were evaluated by agarose gel retardation assay. Gel electrophoresis experiments were performed using 4.5-5% (w/v) agarose gels containing 0.5 µg/ml ethidium bromide in a 1× Tris–boric acid–EDTA (TBE) buffer solution. The samples were run at 70 V for 60-75 min and the bands were visualized under UV illumination by UVIngenius LHR Gel Imaging System.

The zeta-potential and size analyses were performed by preparing dendriplexes at 1×, 3× and 6× ratio of dendrimer/miRNA (w/w). 1×dendrimer/miRNA (w/w) ratio was determined by examining gel retardation assay results. Dendriplexes were prepared approximately 1h before the measurement and just prior to measurement mixed in

ultrapure water, subsequently filtered through a Millex-AP Syringe Filter (EMD Millipore). The zeta-potential and size of the dendriplexes were measured by using Zetasizer Nano ZS-90 (Malvern Instruments Ltd, UK) at 25 °C in the folded capillary zeta cell (Malvern) and disposable polystyrene cuvettes, respectively.

Transmission electron microscopy (TEM) images were also obtained in order to examine morphology and size of the dendriplexes. 6×dendrimer/miRNA (w/w) dendriplexes were prepared in DNase/RNAse free water at room temperature and incubated for 30-60 min. For TEM observation 10 µl of the prepared dendriplex solution was dropped on the carbon-coated copper grid and dried for 60-90 min at room temperature. TEM images of the dried specimens were obtained by using FEI Tecnai G2 220 kVtransmission electron microscope at an acceleration voltage of 120 kV.

Cell culture and miRNA transfection

MRC-5 (human lung fibroblast cell line, ATCC® CCL-171™) cells were used to evaluate the transfection efficiency and cytotoxicity of the synthesized fluorinated dendrimers. The cells were cultured in 0.1% gelatin-coated plates and maintained in FibroGRO™ Complete Media Kit (EMD Millipore) at 37 ⌗C and 5% CO_2. No antibiotics were added to the culture media. Cells were passaged with TrypLE™ Express Enzyme (Gibco).

The transfection experiments were performed with Dy547-labelled miRNA (miRIDIAN microRNA Mimic Transfection Control with Dy547, Dharmacon). MRC-5 cells were cultured in 0.1% gelatin-coated 24-well plates 24 h prior to the transfection. 10 pmol miRNA was mixed with fluorinated dendrimers at 1x, 3x and 6x dendrimer/miRNA (w/w) ratios and the dendriplexes were incubated for 30-60 min. Prepared dendriplexes were dropped to the wells and incubated for 8 h. Then, the media were replaced with fresh media and the cells were incubated for further 48 h at 37 °C and 5% CO_2.

Transfection efficiencies of the fluorinated dendrimer/miRNA dendriplexes

Transfection efficiencies were evaluated by flow cytometer analysis and also intracytoplasmic localization of the fluorinated dendrimer/miRNA dendriplexes were observed by confocal laser scanning microscopy. The transfection experiments were conducted as described above. At the end of 8 h transfection period the media were removed. After washing with PBS twice, the cells were detached with TrypLE™ Express Enzyme and then centrifuged for 4-5 min at 200 g. The cells were resuspended in PBSand analyzed by using CytoFLEX Flow Cytometer and CytExpert 1.2 software.

For confocal imaging, at the end of incubation period the media were removed, and the cells fixed with 4% paraformaldehyde solution in PBS for 15 min at 37 C⌗. Then, cells were stained with 5 µg/ml WGA conjugate solution in PBS and 1:4000 Hoechst 33342 dye. Images were obtained by using LSM 780 NLO Multi Photon and Confocal Microscope (Zeiss, Germany).

Cytotoxicity of the fluorinated dendrimer/miRNA dendriplexes

Cytotoxicity of the transfections made by the fluorinated dendrimer/miRNA dendriplexes was evaluated by XTT [2,3-Bis(2-methoxy-4-nitro-5-sulfonyl)-2H-tetrazolium-5-carboxanilideinner salt] method. Cell proliferation kit, XTT based (Roche) was used according to the instructions of the manufacturer. Briefly, MRC-5 cells were cultured in 96-well plates at a density around 6×103 cells per well overnight. The transfections were conducted as described above. At the end of 8 h transfection period, the media were removed, and the cells were incubated for further 48 h at 37 ⌗C and 5% CO2. Then, reaction solution containing XTT reagent and activation reagent was added to the wells and incubated for further 8 h at 37 ⌗C and 5% CO2. Absorbance of the each well was detected at 450 nm by a Synergy H1 Hybrid Multi-Mode Reader.

Molecular docking calculations

In order to get optimized structure of G5-PFB and G5-PFP, molecular mechanics calculations were performed by Polak-Ribiere algorithm (conjugated gradient) with root mean square (RMS) gradient of 0.010 kcal/(Åmol). Duplex miRNA was generated by Nucleic Acid Builder (http://casegroup.rutgers.edu/). In order to calculate atomic contact energy (ACE) values PatchDock Beta 1.3 Version program was used (https://bioinfo3d.cs.tau.ac.il/PatchDock). A RMS deviation tolerance for each docking was set at 8.0 Å. The remaining parameters were set as default. Quantitative structure-activity relationship (QSAR) calculations were used to obtain topological polar surface area (TPSA) values. ACE and TPSA values were evaluated for comparisons.

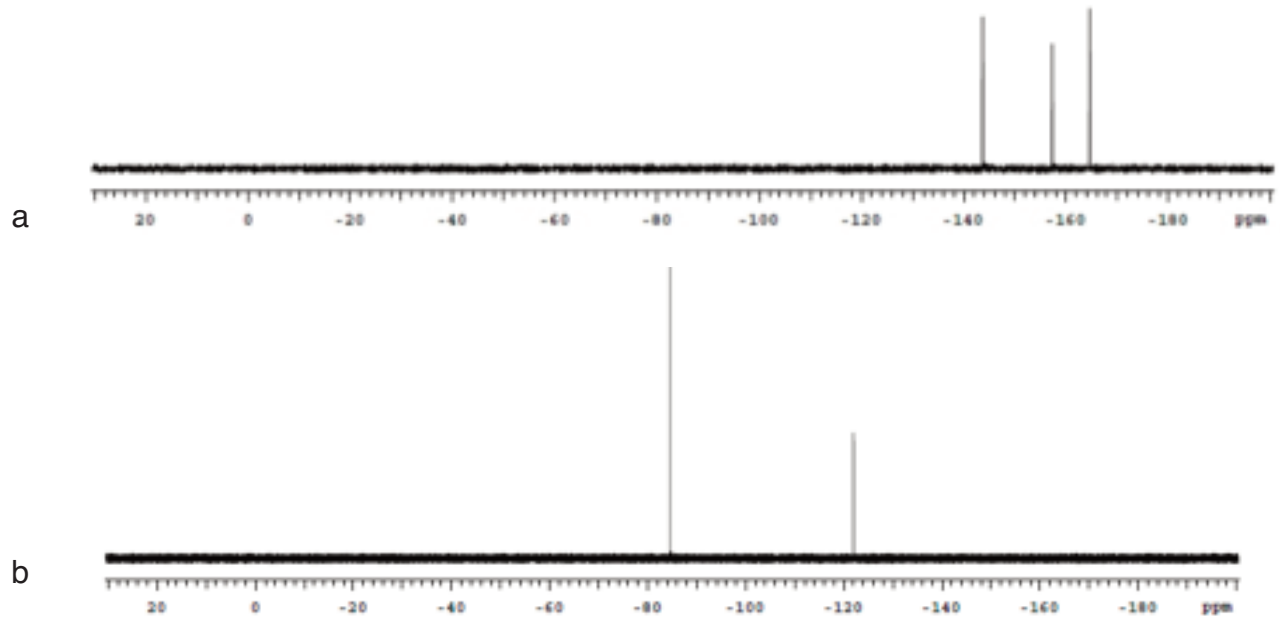

Figure 1. ^{19}F-NMR spectra of G5-PFB (a) δ ppm, CDCl3: -164.61, -157.22, -143.66 and G5-PFP (b) δ ppm, $CDCl_3$: -121.95, -84.56.

Statistical Analysis

The data were given as mean (SD) and analyzed by One Way ANOVA Test (with Bonferroni Corrected).

Results

Synthesis and characterization of dendrimers

Synthesis of G5 PAMAM was performed by adding methanolic solutions of methylacrylate and ethylenediamine into cystamine until to get to G5 by iterative reactions of Michael addition and exhaustive amidation. Presence of the -NH2 and other functional groups on the G5 PAMAM dendrimer surface and -CH2-groups on the dendrons were confirmed by FT-IR and 13C-NMR analysis, respectively. FT-IR (ATR, 4000-450 cm-1): 3270 (o), 3050 (o), 2925 (o), 2835 (o), 1650 (k), 1556 (k), 1460 (k), 1150 (o), 1030(o). 13C-NMR (D2O, 400 MHz, δ ppm): 32.6, 37.8, 38.7, 41.8, 42.3, 47.9, 49.1, 51.5, 177.3.

PFB and PFP were conjugated to the surface of G5 PAMAM dendrimer using anaddition reaction. The number of PFB and PFP moieties modified on each G5 dendrimer was calculated using ^{19}F-NMR analysis (Figure 1). 2,2,2-trifluoroethanol was used as an internal standard. For both fluorinated dendrimer, 25% of the -NH2 groups on the surface were found to be fluorinated.

Characterization of dendriplexes

The ability of fluorinated dendrimers to complex with miRNA as a function of dendrimer/miRNA weight ratio was evaluated by gel retardation assay. The amount of miRNA was kept constant at 10 pmol. As shown in Figure 2, complete complexation of

Table 1. Size and zeta potential of dendrimer/miRNA dendriplexes

Dendrimer	w/w	Z-Average, (d.nm)	Zeta potential, (mV ± SD)
G5-PFB	1×	223.5	36.4 ± 16.1
	3×	199.2	60.5 ± 8.49
	6×	191.2	76.1 ± 10.7
G5-PFP	1×	256.6	53.4 ± 9.33
	3×	201.3	52.3 ± 20.7
	6×	192.5	78.8 ± 12.4

G5 = generation five, PFB = pentafluorobenzoic acid, PFP = pentafluoropropionic acid

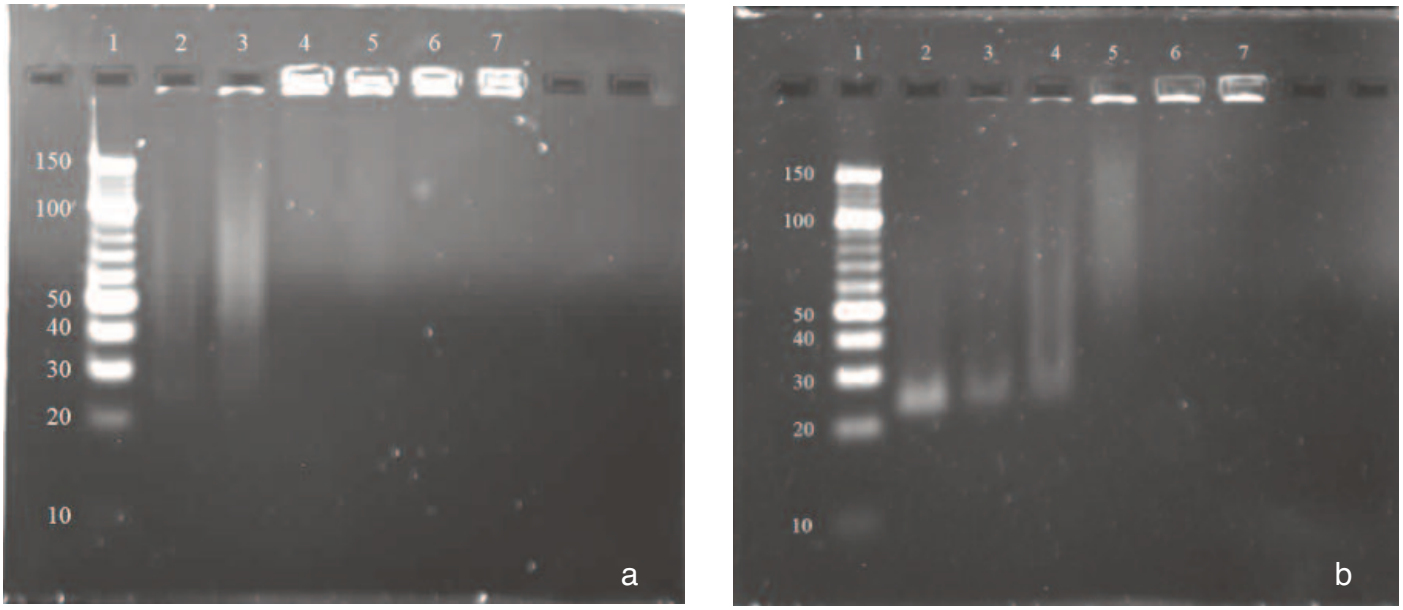

Figure 2. Agarose gel retardation assay of dendriplexes(a) G5-PFB/miRNA (w/w): Line 1- 10 bp DNA Ladder; 2- 3.75:1; 3- 7.5:1; 4- 15:1; 5- 20:1; 6- 40:1; 7-, 80:1; (b) G5-PFP/miRNA (w/w): Line 1- 10 bp DNA Ladder; 2- 0.7:1; 3- 1.4:1; 4- 3.5:1; 5- 7:1; 6- 14:1; 7-21:1.

miRNA was observed for G5-PFB and G5-PFP at a w/w ratio of 20 and 14, respectively.

After having demonstrated that fluorinated dendrimers are able to complex with miRNA, the characteristics of these dendriplexes at 1×, 3× and 6× dendrimer/miRNA (w/w) ratios were evaluated by size and zeta potential measurements. The results of size and zeta potential analysis for dendriplexes of G5-PFB and G5-PFP with miRNA are given in Table 1. Particle size analysis showed the formation of ~200 nm dendriplexes. Despite the slight changes of size with the w/w ratio, evaluation of the surface charges of the dendriplexes showed the significant increases with increasing w/w ratios. The highest charge was observed at w/w ratio of 6× which was 76-78 mV for both G5-PFB and G5-PFP.

Since the method used for particle size measurement, which is dynamic light scattering, detects light scattering and does not measure real particle size, morphology and the particle size of dendriplexes were also evaluated by TEM. As shown in Figure 3, dendriplexes formed spherical structures with mean particle size between 120-190 nm and 80-130 nm for G5-PFB and G5-PFP, respectively.

Cytotoxicity of dendriplexes

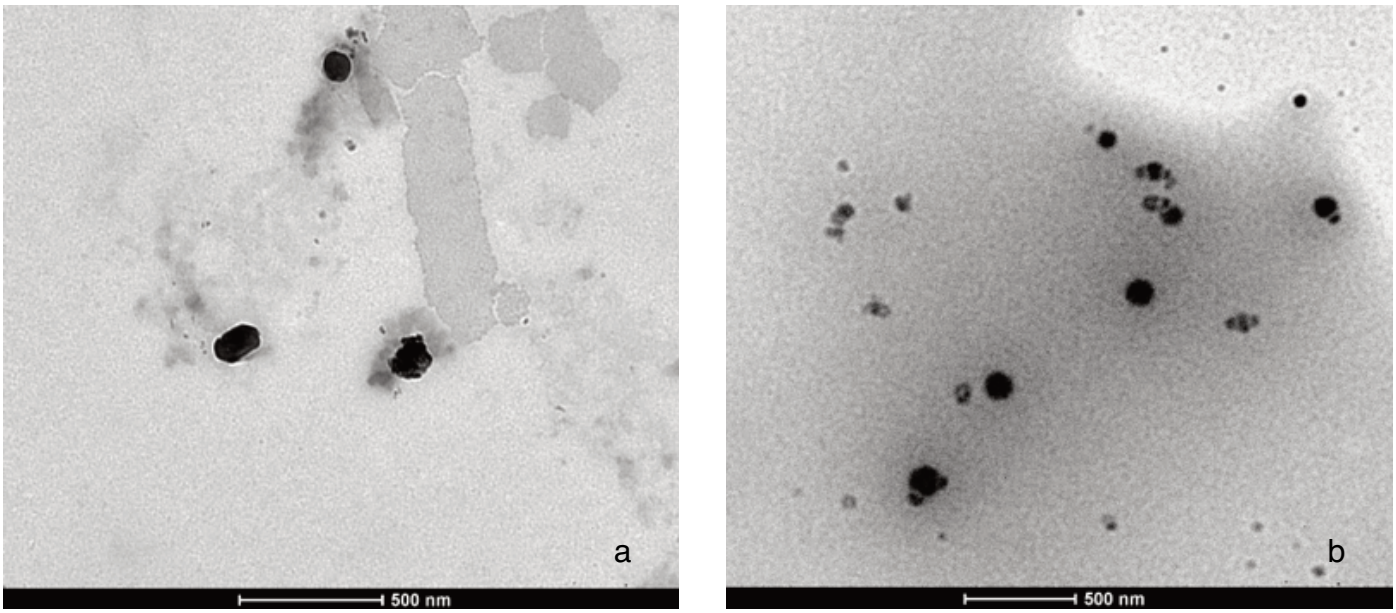

Figure 3. TEM images of G5-PFB/miRNA (a) and G5-PFP/miRNA (b) dendriplexes.

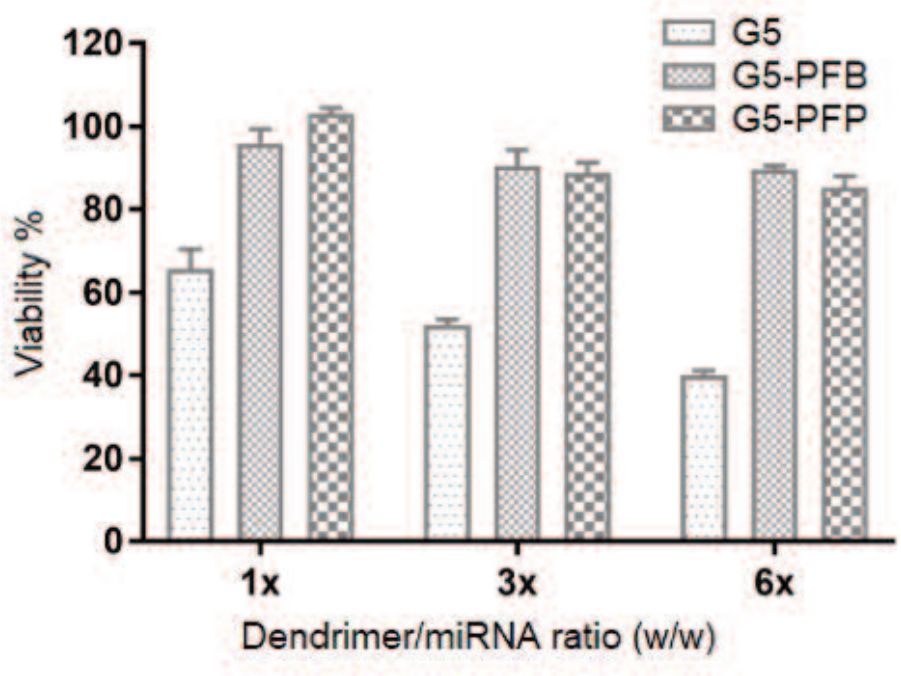

Figure 4. Cytotoxicity of dendrimer/miRNA dendriplexes.

The cytotoxicity of G5-miRNA, G5-PFB/miRNA and G5-PFP/miRNA dendriplexes in MRC-5 cells was evaluated by XTT assay. A seen in Figure 4, fluorination has increased the viability and both of the fluorinated dendrimers showed minimal cytotoxicity on MRC-5 cells. When G5-PFP and G5-PFB compared with each other, while there was not a statistical significance for 3× ratio (p = 0.438), there was a statistical significance for 1× (p = 0.003) and 6× (p = 0.004).

Transfection efficiencies of dendriplexes

Dy547-labeled miRNA uptake by G5-PFB and G5-PFP dendrimers was evaluated by flow cytometry. The percentage of Dy547 positive cells was used as a measure of transfection efficiency. G5-PFB, showed 4.57%, 44.5% and 68.6% efficiency for w/w ratios of 1×, 3× and 6×, respectively. For G5-PFP, transfection efficiencies were 8.77%, 75.4% and 90.7% for w/w ratios of 1×, 3× and 6×, respectively (Figure 5). G5-PFP showed superior efficiency than G5-PFB as a transfection reagent.

In order to show cellular distribution of the miRNAs delivered by fluorinated dendrimers confocal imaging was also performed. Images were obtained for dendriplexes at 6× dendrimer/miRNA (w/w) ratio. As seen in Figure 6, bright green fluorescent signals of Dy547 were localized to cytoplasm.

Effect of structural differences over transfection efficiency

To compare two fluorinated dendrimers with each other Patchdock tool was used. Patchdock is an algorithm for molecular docking that identifies docking transformations, molecular shape complementaritiesand can also perform clustering and calculates the global binding energy [16, 17]. TPSA and ACE values obtained from QSAR and molecular docking calculations are given in Table 2. TPSA values of the both dendriplexes were so close to each

Table 2. The QSAR and docking results of dendriplexes

Dendriplex	Pose Score	ACE (kcal/mol)	TPSA (Å^2)
G5-PFB/miRNA	15228	-2815.46	12041
G5-PFP/miRNA	14840	-2099.33	12054

ACE = atomic contact energy, G5 = generation five, miRNA = microRNA, QSAR = quantitative structure-activity relationship, PFB = pentafluorobenzoic acid, PFP = pentafluoropropionic acid, TPSA= topological polar surface area

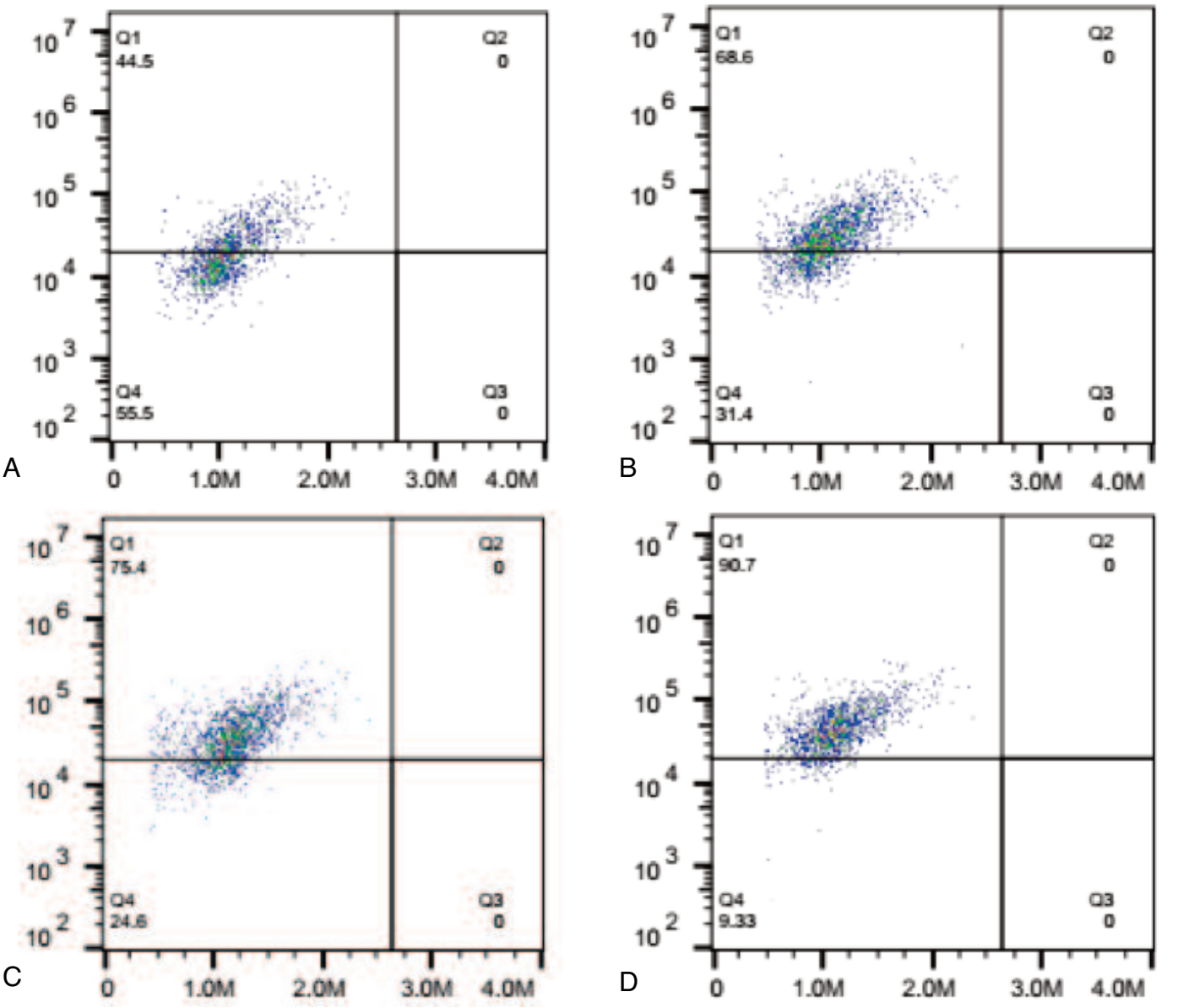

Figure 5. Transfection efficiencies of G5-PFB 3× (a), G5-PFB 6× (b), G5-PFP 3× (c) and G5-PFB 6× (d).

other. G5-PFP showed good ACE, -2099 kcal/mol, with the miRNA than G5-PFB.This means that, G5-PFP formed stronger complexation with miRNA and therefore G5-PFP/miRNA dendriplex achieved high transfection efficiency than G5-PFB/miRNA. This difference was attributed to the molecular structure of the fluoro-compounds (Figure 7).

Discussion

In this study, aliphatic (PFP) and aromatic (PFB) two fluoro compounds were used for PAMAM dendrimer modifications. When the structures given in Figure 7 taken into the account, due to chemical environment differences of the fluorines, two and three peaks are expected in the ^{19}F-NMR spectrums of the PFP and PFB, respectively. The ^{19}F-NMR spectra of the products confirmed the addition of PFB and PFP to the surface of G5 PAMAM.

Gel retardation analysis showed the fluorinated dendrimers miRNA complexation ability. Size measurement and TEM analysis also revealed that G5-PFB and G5-PFP can condense the miRNA in to the dendriplexes below 200 nm at w/w ratios above 1×. Size of the dendriplexes is one of the factors for effective delivery of miRNA/siRNA to the cells. Studies suggest that polyplexes below or as large as 200 nm are the optimal size for the non-viral vectors because they can pass through the cell membrane by receptor-mediated endocytosis [18-20]. Zeta potential measurements of dendriplexes showed significant increases with increasing dendrimer concentration. It was 76-78 mV for G5-PFB and G5-PFP at w/w ratio

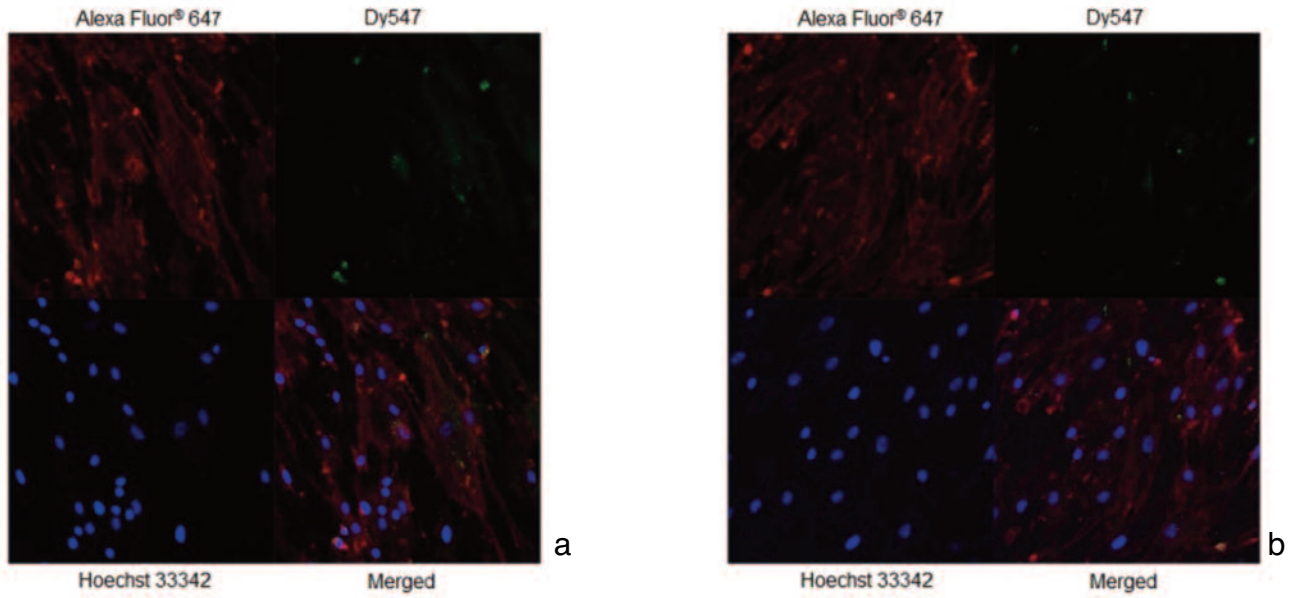

Figure 6. Confocal images of MRC-5 cells transfected with G5-PFB/miRNA (a) and G5-PFP/miRNA (b) dendriplexes. Plasma membranes were stained WGA Alexa Fluor® 647 conjugate and the nuclei were stained with Hoechst 33342. miRNA was labeled with Dy547.

of 6×. Zeta potential is a measure of the effective electric charge on the nanoparticle surface and provides information about particle stability. The higher magnitude of zeta potentials exhibits increased stability and protects them from aggregation. Positive charge of particles also facilitates the transport of target into the cell and increases their solubility in the aqueous environment [21, 22].

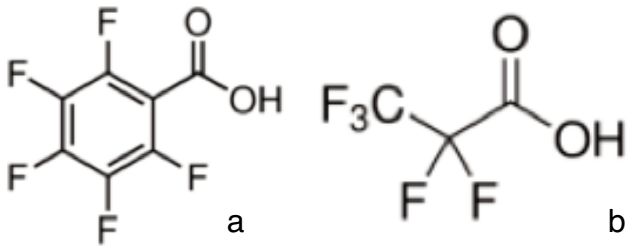

Figure 7. Structures of PFB (a) and PFP (b) molecules.

The relation between surface cationic charges and cytotoxicity is well known and has been mentioned for various cell lines in culture [23, 24]. In our results, similar relation was observed for MRC-5 cells and reduction of the surface cationic charges with fluorination has increased the cell viability. Despite dose-dependent cytotoxicity by increasing dendrimer concentrations, the cells treated with all dendriplexes showed cell viability above 85%.

To evaluate the transfection efficiencies of prepared fluorinated dendrimers, flow cytometry analysis was conducted with Dy547-labeled miRNAs. Results showed dose-dependent efficiency increase with increasing dendrimer concentrations. It was 68.6% and 90.7% at w/w ratio of 6× for G5-PFB and G5-PFP, respectively. To explain the difference in efficiency of fluorinated dendrimers molecular docking and QSAR calculations were used. Proximity of TPSA values of dendriplexes points out their almost equality of cationic surface charges and smooth penetration capability into the cell. High pose rate and low ACE value of G5-PFP/miRNA dendriplex shows its highly stable complexation with miRNA (Table 2). On the other hand, aliphatic structure of G5-PFP leads less intramolecular interactions than aromatic structured G5-PFB. Therefore, it was concluded that higher transfection efficiency of G5-PFP was because of its structure and more stable complexation with miRNA.

Conclusions

High transfection efficiency and low cytotoxicity during transfection are the two main factors influencing cationic polymer mediated-nucleotides delivery [25]. In this study, two fluorinated dendrimers were synthesized and their miRNA delivery efficiencies were evaluated on MRC-5 cells. The fluorinated PAMAM dendrimers showed efficient miRNA complexation ability and high cell viability above 85%. In addition, PAMAM dendrimer fluorinated with PFP performed superior transfection efficiency than PFB modified. It was concluded that

fluoroaliphatic compounds such as PFP can be used for surface modifications of dendrimers. Also, the structure-function relationship seen in this study will be helpful for other dendrimer modification studies in the future.

Authorship declaration

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

Author contributions

A.O. performed dendriplex characterization, cytotoxicity, transfection efficiency experiments, data analysis and also designed the study and wrote the manuscript. H.N. performed the synthesis and characterization of dendrimers, made the theoretical calculations and wrote the manuscript.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Effect of functional endoscopic sinus surgery on asthma control with nasal polyposis and chronic rhinosinusitis

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ABSTRACT

Objectives. The purpose of this study is to examine the effect of endoscopic sinus surgeons on the control of disease in asthma combined with chronic rhinosinusitis. ***Methods.*** Twenty-three asthma patients with chronic rhinosinusitis were evaluated retrospectively. They were assessed with asthma control test and pulmonary function tests. Subjective nasal obstruction assessment was done according to the nasal obstruction symptoms evaluation (NOSE) scale. Other subjective sinonasal complaints were classified using visual analogue scale (VAS). At postoperative third month, pulmonary function tests, asthma control test, NOSE and VAS were reevaluated. ***Results.*** According to asthma control test, number of patients under control, partial controlled and uncontrolled were 2/6/15 preoperatively and 14/4/5 postoperatively, respectively. There was permanent or partial improvement in fullness in the nose sniffle of 95.7% according to NOSE. The most remarkable permanent improvement in the symptoms were seen in the feeling of pressure in the face of 75%, smell disorders of 66.7% according to sinonasal visual analogue scale assessment. NOSE and VAS values were significantly improved postoperatively (<0.05). ***Conclusions.*** Uncontrolled asthma associated with chronic rinosinusitis can benefit from functional endoscopic sinus surgery in asthma control level, nasal blockage symptoms and other sinonasal complaints.

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Keywords: asthma, endoscopic sinus surgery, chronic rhinosinusitis, asthma control test

Introduction

Asthma is a chronic inflammatory disease of the airways. In most patients it affects all airways, including the upper respiratory tract and the nose [1]. Chronic rhinosinusitis is associated with more severe asthma. It is an inflammatory condition of the paranasal sinuses. It distincts two clinically entities: Chronic rhinosinusitis without nasal polyposis and chronic rhinosinusitis with nasal polyposis [2]. Treatment of sinusitis in patients with asthma can be improved of patients' lower airway disease. Surgical approaches have yielded good results in the treatment of chronic sinusitis [3].

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In this study, we evaluated the subjective sinonasal complaints, changes in objective pulmonary function tests and asthma control test, following functional endoscopic sinus surgery (FESS) applied for cases with chronic rhinosinusitis and asthma components.

Methods

Patients

This study involved 23 patients admitted to our clinics with chronic rhinosinusitis resistant to retrospective medicine who had been treated for asthma and operated with functinal sinus surgery. Informed consent was obtained from participants for all surgical procedures.

Exclusion criteria were being smoker, systemic diseases (cystic fibrosis, bronchiectasis, chronic obstructive pulmonary disease, diabetes, neoplasia, fungal sinusitis etc.), aspirin hypersensitivity (Samter's Syndrome), previous nasal operation and having upper respiratory infection in the last month.

Asthma Control Test

Their clinical conditions in terms of asthma were assessed preoperatively and at the 3rd month postoperatively with asthma control test [2, 4].

Spirometry

Pulmonary function tests were performed spirometrically (Spirothor Wavefront); FVC (forced vital capacity), FEV1 (forced expiratory volume in the first second) and FEV1/FVC preoperatively and at the postoperative 3rd month.

The Nasal Obstruction Symptoms Evaluation (NOSE) Scale

Subjective nasal obstruction assessment was done according to NOSE scale. The components of NOSE are nasal congestion and sniffing, nasal blockage or obstruction, trouble breathing through nose, trouble sleeping and unable to get enough air through nose during exercise or exertion, scored as, 0: not a problem, 1: very mild problem, 2: moderate problem, 3: fairly bad problem, 4: severe problem [5].

Visual Analogue Scale (VAS)

Other subjective sinonasal complaints (postnasal drip, headache, feeling of pressure in the face, epistaxis, smell disorders, taste disorders and chronic cough) were classified using VAS carts (visual analogue scale) as 0: no symptom, 1: lightly tolerable, 2: moderate still tolerable 3: serious symptom restricting daily activity [6].

Preoperative Period

In the preoperative period, antibiotherapy (Klaritromycine 500 mg BID) for 2-4 weeks, topical intranasal steroid (budesonide 400 mcg/day for 8 weeks, antihistamine (levocetirizine 10 mg/day) and oral systemic steroid (methylprednisolone, 1 mg/kg/day) were given to the patients with nasal polyps; oral methylprednisolone treatment was discontinued gradually before the operation. Functional endoscopic sinus surgery was performed under general anesthesia.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) (IBM Corp. Released 2015. IBM SPSS Statisticsfor Windows, Version 23.0. Armonk, NY: IBM Corp.). Numerical values were expressed by the mean and the standard deviation; and their distribution was tested with the Kolmogorov-Smirnov test. Comparison of pulmonary function tests, asthma control test, NOSE and VAS were assessed preoperatively and postoperatively with Wilcoxon test and $p<0.05$ was accepted as level of significance.

Results

This retrospective study included 23 patients (13 male, 10 female; age range: 17-63, mean age: 38.4 ± 13.4) with chronic rhinosinusitis and asthma resistant. Mean asthma duration was 5.8 ± 3.0 years. Mean FEV1, FVC and FEV1/FVC were 74.22% ± 11.90; 70.78% ± 10.37 and 87.17% ± 5.70 preoperatively and 87.55% ± 9.36; 87.81% ± 9.95 and 83.02% ± 7.78 postoperatively, respectively. Mean FEV1 and FVC increased significantly three months after operation ($p<0.001$), however mean FEV1/FVC reduced significantly ($p=0.013$) (Figure 1).

While two (8.7%) patients were clinically under control, 6 (26.1%) partial controlled and 15 (65.2%) were not under control according to the asthma control test preoperatively. After three months, 14 (60.9%) were under control, 4 (17.4%) partial controlled and 5 (21.7%) were uncontrolled (Figure 2). There was statistically significant improvement in postoperative asthma control test ($p=0.001$).

Postnasal drip, headache, feeling pressure,

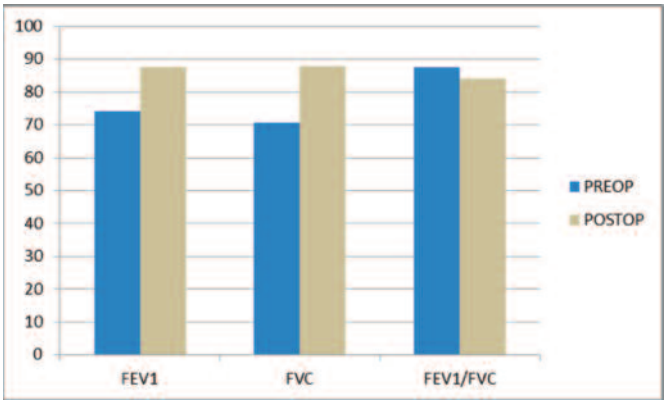

Figure 1. Preoperative and postoperative pulmonary function test results. FEV1 = forced expiratory volume in the first second, FVC = forced vital capacity

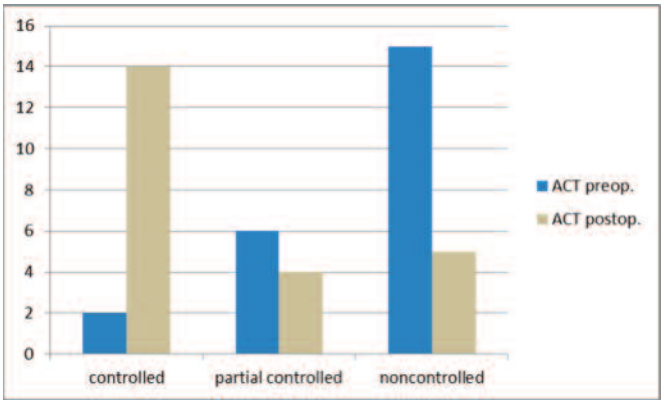

Figure 2. Preoperative and postoperative ACT values. ACT = asthma control test

epistaxis, smell disorders, taste perversion and chronic cough have improved in postoperatively in NOSE (p<0.001, p=0.001, p<0.001, p=0.046, p<0.001, p=0.007, p=0.003, respectively). Postnasal blockage, stuffiness, nasal discomfort during breahting, sleep difficulties, difficulty breathing after physical activity improved statistically significantly postoperatively (p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, respectively).

The most remarkable permanent or partial improvement in the symptoms were seen in the feeling of pressure in the face of 100%, smell disorders of 100% and headache of 87.5% according to NOSE teste (Figure 3). There was permanent or partial improvoment in fullness in the nose sniffle (95.7%), stuffiness (86.9%), nasal discomfort while breathing (91.3%) sleep difficulties (69.6%), difficulty breathing after physical activity (56.5%) according to sinonasal VAS assessment (Figure 4).

Discussion

In the present study we investigated the possible effects of functional endoscopic sinus surgery at asthma control in patients who have uncontrolled asthma with chronic rhinosinusitis. This study showed that the number of controlled patients increased from 2 (8.7%) preoperatively to 14 (60.9%) postoperatively, and the number of uncontrolled patients reduced to 5 (21.7%) from 15 (65.2%). There was a remarkable improvement in clinical control levels of our patients in terms of asthma at the 3rd month after functional endoscopic sinus surgery.

In the study of Proimos *et al.* [7], controlled patients were 17.4% to 16.3%, partial controlled patients were 74.4% to 83.7%, and not under controlled patients were 8.1% to 0% changed postoperatively at the sixth month. The rate of not controlled asthmatic patients who were operated on in

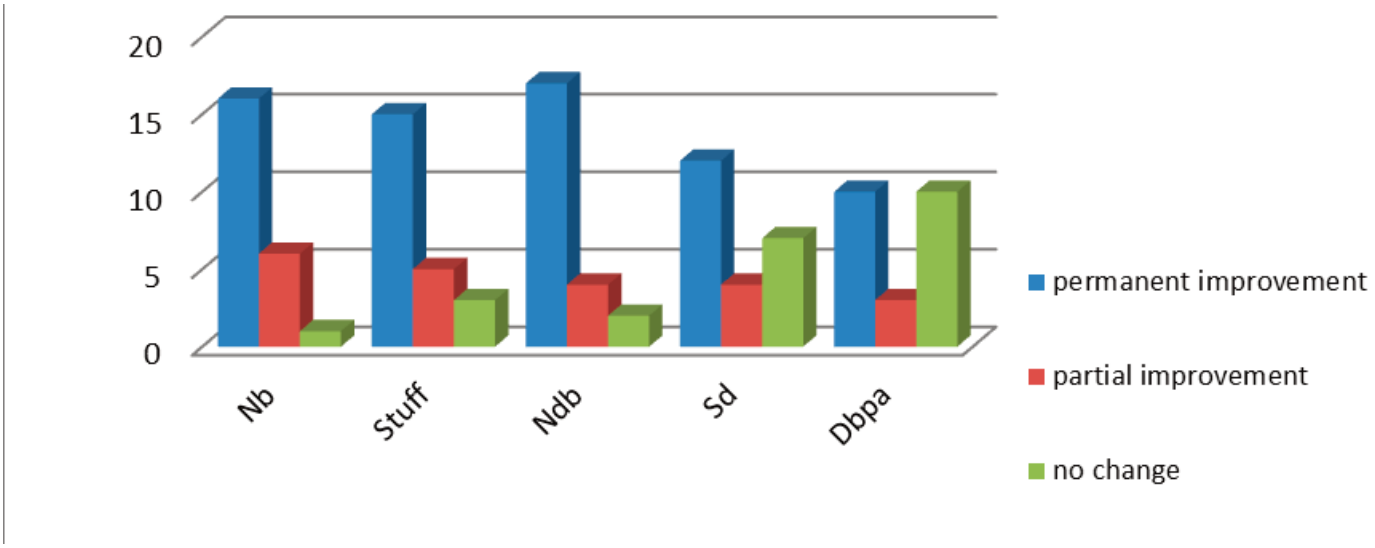

Figure 3. Postoperatively improvement according the NOSE test. NOSE = nasal obstruction symptoms evaluation, Nb = nasal blokage, Stff = stuffiness, Ndb = nasal discomfort while breathing, Sd = sleep difficulties, Dbpa = difficulty breathing after physical activity

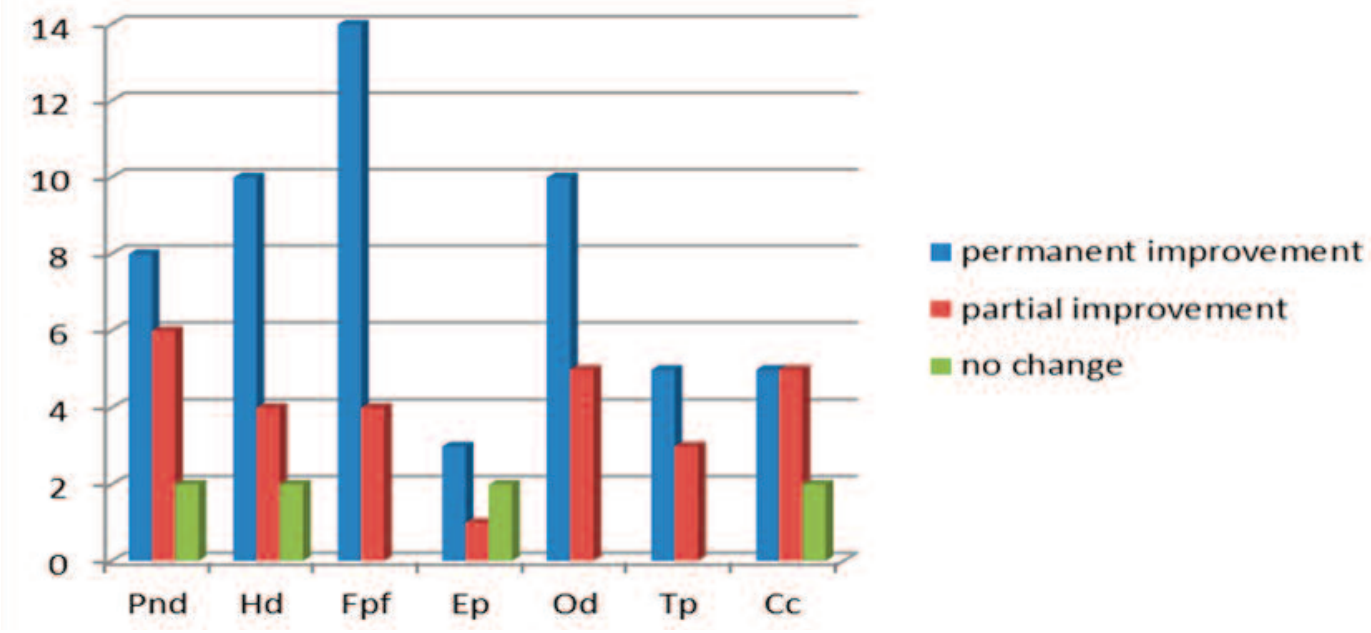

Figure 4. Sinonasal symptoms improvement according VAS. VAS = visual analogue scale, Pnd = postnasal drip, Hd = headache, Fpf = feeling of pressure in the face, Ep = epistaxis, Od = olfactory disorders, Tp = taste perversion, Cc = chronic cough

this study was much lower than our study. Dejima *et al.* [8] have found a remarkable improvement in subjective complaints, daytime and nighttime asthma symptoms in out of 75% of 19 patients with asthma. However, no change in the asthma symptom score was found in Goldstein *et al.*'s study [9]. Chen *et al.* [10] have obtained significant improvement in asthma control levels in their study of 27 cases. Ragab *et al.* [11] assessed prospectively 43 patients with asthma in a randomized study comparing the effects of sinus surgery and medical treatment for chronic rhinosinusitis with nasal polyps. Asthma control improved significantly after both treatments. Dunlop *et al.* [12] have reported that, 40% of 50 chronic rhinosinusitis patients had an improvement in their asthma after 12 weeks following endoscopic sinus surgery, while 54% had no change, and in 6% clinical control was assessed as worse.

We have determined a significant postoperative improvement in FVC and FEV1 values, compatible with Dejima *et al.*'s study [8]. But mean FEV1/FVC reduced significantly ($p = 0.013$). We have found it consistent that there was an improvement in both restrictive and obstructive types of respiratory failure after functional endoscopic sinus surgery. Gulati *et al.* [3] reported improvement in FEV1 measurements. Chen *et al.* [10] did not found any significant difference in terms of asthma drug consumption and pulmonary function tests postoperatively. However, in other study, they were found improvement in the pulmonary function and asthma control test at one year after surgery [13]. In Goldstein *et al.*'s study [9], the results of the pulmonary function test had not changed. In our study, there was improvement in NOSE and VAS evaluations postoperatively. We had determined improvement in nasal blockage at a ratio of 69.6% according to NOSE. According to VAS, we have determined the highest permanent improvement of 75% in feeling of pressure in the face, 66.7% smell function and 62.2% in headache. Uri *et al.* [14] have not report an improvement in asthma symptoms after endoscopic sinus surgery, whereas quality of life had improved. Philips *et al.* [15] showed that the most common and also recoverable symptom after endoscopic sinus surgery is headache. Chen *et al.* [13] found significant improvement in the VAS score at one year after surgery. Proimos *et al.* [7] found statistically significant improvement after operation in nasal blockage, smell, facial pain and headache. Our findings were consistent with other studies.

We could not find any other study involving all of the findings we evaluated with NOSE and VAS.

The Limitations of the Study

The main limitations of this study have been small sample group. We can follow cases for a short period of 3 months after the operation. Further studies may be necessary to investigate the relationship of the asthma and surgical treatment of chronic rhinosinusitis as functional endoscopic sinus surgery that it can be suggested to eliminate the risk factor of asthma control.

Conclusions

Despite of low in case number, our hypothesis that asthma control can be increased, especially in cases of chronic rhinosinusitis with nasal polyps, may be defended. The improvement in sinonasal symptoms by surgical treatment of nasal blockage, may provide the clinical control of asthma.

Authorship declaration

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The frequency of toll-like receptor 4 gene polymorphism in ankylosing spondylitis and its relationship between disease activity

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ABSTRACT

Objective. We aimed to evaluate the frequency of toll-like receptor 4 (TLR4) gene polymorphism and its relationship between disease activity in patients with ankylosing spondylitis (AS). ***Methods.*** Forty-one AS patients (25 male/16 female) fullfilling the 1984 Modified New York Criteria and 41 healthy controls (25 male/16 female) were included in this study. Disease activity of the AS patients was assesed by Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). The TLR4 gene polymorphism of AS patients and healthy controls were analyzed by Real-Time Polymerase Chain Reaction (PCR) System. ***Results.*** Three (7.3%) patients with AS had TLR4 gene polymorphism compared with healthy controls (0/41; 0%). Two of these patients had heterozygous mutation and one had homozygous mutation. Significant correlation was not found between TLR4 gene polymorphism and BASDAI score ($p > 0.05$). ***Conclusions.*** In our study, TLR4 gene polymorphism was higher in patients with AS compared with control group. But, this polymorphism was not associated with disease activity, erythrocyte sedimentation rate and C-reactive protein levels.

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Keywords: Ankylosing spondylitis, disease activity, mutation, polymorphism, toll-like receptor 4

Introduction

Ankylosing spondylitis (AS) is an autoimmune, chronic inflammatory disease which is characterized by axial skeletal ankylosis, inflammation at the entheses and arthritis of the peripheral limbs [1]. Although the exact cause of AS remains unclear, the role of genetic susceptibility and epigenetic modifications caused through environmental factors have been respected in the pathogenesis [2]. Toll-like receptors (TLRs) defined as type I integral membrane glycoproteins play important roles in innate immune system [3]. TLR ligands are released from inflammatory cells and activates the TLR signaling pathway. With the activation of this pathway; cytokine, growth factors and anti-apoptotic proteins are expressed. Tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, IL-12, IL-18, etc., and some

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other proinflammatory cytokines are activated with the gene activation [4].

In our study we investigated the frequency of TLR 4 (TLR4) gene polymorphism and its relationship between disease activity in patients with AS.

Methods

The study was carried out at Department of Physical Medicine and Rehabilitation, Çanakkale Onsekiz Mart University. The ethics Committee of the Institution approved the study. Written informed consent was taken from all participants. Fourty-one AS patients who were diagnosed with AS according to the 1984 Modified New York criteria and 41 healthy controls age and sex-matched were included in this study. Patients with AS and healthy controls were not taking any treatment. Healty controls were selected from hospital staff. Exclusion criteria for the study were presence of additional co-morbidities (rheumatologic diseases, malignities, systemic diseases, infections, neuromuscular diseases, etc.).

The all medical records of these patients in our hospital were analysed and later ages, gender, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score were recorded to the research form. All blood samples were taken for hemogram, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Hemogram parameters were stored in the tubes in which ethylene diamine tetra acetic acid was used as anticoagulant. ESR (normal range: 0-20 mm/h) was measured by Westergren method [5, 6] (Eventus Vacuplus ESR100, Turkey), CRP (normal range: 0-5 mg/L) was measured by immunoturbidimetry [7] (Prestige 24i CRP Ultra, P.Z. Cormay, Lublin, Poland).

About 10 ml of venous blood were obtained from each subject for TLR4 gene polymorphism and then stored at 4 oC until analysis. For TLR4 gene polymorphism analysis, total cellular RNA was extracted using the High Pure RNA Isolation Kit (Roche, Germany) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from the RNA using the Magna Pure Compact Isolation Kit (Roche, Germany) and Invitek Kit (Roche, Germany) according to the manufacturer's instructions [8]. HLA-B27 was determined by PCR [9] and TLR4 gene was genotyped by Real Time Polimerize Chain Reaction (PCR) using the TaqMan Gene Expression Assays containing the FAM dye-labeled probes (TaqMan Pre-designed Gene Expression Products, Applied Biosystems, Foster City, CA, USA) and Light Cycler Probes Master 480 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) [10]. After PCR analysis A and G alleles of TLR4 were determined using melting point analysis [11].

Statistical Analysis

TLR4 A896G gene polymorphism and the frequency of allele and genotype was tested by descriptive statistics in AS patints and healty control groups. To compare the association between TLR4 gene polymorphism and HLA-B27, Fisher's exact test was used. Kolmogorov-Smirnov test was used to determine whether sample data is normally distributed. The Mann-Whitney nonparametric test was used to determine the difference between ESR and CRP in TLR4 gene polymorphism positive and negative groups. Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS), version 19.0 (IBM Corp; Armonk, NY, USA) and $p < 0.05$ was considered to be statistically significant.

Results

There were 25 males and 16 females in the both groups. The mean age was 40.24 ± 9.44 years (range; 25-60 years) for AS group and 40.76 ± 8.41 (range; 27-57) for healty controls. There were no significant difference between groups in terms of age, and gender. Three (7.3%) patients with AS had TLR4 gene

Table 1. The frequency of toll-like receptor 4 gene polymorphism

TLR4 gene polymorphism	AS group n (%)	Healty controls n (%)
Positive	3 (7.3)	0 (0)
Negative	38 (92.7)	41 (100)
Total	**41 (100)**	**41 (100)**

TLR4 = toll-like receptor 4, AS = ankylosing spondylitis

Table 2. The association between TLR4 gene polymorphism and HLA-B27

HLA-B27	TLR4 gene polymorphism Negative n (%)	Positive n (%)	*p* value
Positive	31 (93.9)	2 (6.1)	0.488
Negative	7 (87.5)	1 (12.5)	

TLR4 = toll-like receptor 4

Table 3. The association between TLR4 gene polymorphism and disease activity

	TLR 4 gene polymorphism Positive	Negative	*p value*
ESR (mm/h)	31.82 ± 19.69	34.00 ± 32.08	0.860
CRP (mg/L)	1.17 ± 1.10	1.26 ± 1.02	0.817
BASDAI score	3.46 ± 1.87	2.67 ± 1.32	0.477

Data are shown as mean ± standard deviation. BASDAI = Bath ankylosing spondylitis disease activity index, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, TLR4 = toll-like receptor 4

Table 4. The frequency of allele genotype and the TLR4 A896G polymorphisms in AS patients and control group

TLR4 A896G	AS group (n)	Healthy control group (n)
GENOTYPE		
AA	38	41
AG	2	0
GG	1	0
ALLELE		
A	78	82
G	4	0

TLR4 = toll-like receptor 4, AS = ankylosing spondylitis

polymorphism (2 heterozygous and 1 homozygous) (Table 1) and 2 patients with AS had HLA-B27 positivity (Table 2). In healty controls, there was no TLR4 gene polymorphism and 31 of them had HLA-B27 positivity. Significant difference between groups in terms of TLR4 gene polymorphism and HLA-B27 positivity ($p = 0.488$) (see Table 2).

The association between TLR4 gene polymorphism, ESR, CRP and BASDAI score were presented in Table 3. Significant difference was not found between TLR4 gene polymorphism and ESR ($p = 0.860$), CRP ($p = 0.817$) and BASDAI score ($p = 0.477$). The frequency of allele and genotype and the TLR4 A896G polymorphisms in AS patients and controls were presented in Table 4. Melting point analysis was presented in Figures 1.

Discussion

TLR4 gene polymorphism and its relationship between disease activity in patients with several autoimmune diseases has been investigated in previous studies [12-22]. TLR ligands are primarily involved in innate immune responses [12]. With the activation of the innate immune system proinflammatory cytokines (TNF-α, IL-1 , IL-6, IL-12, IL-18, etc) are expressed [4].

Gergely *et al*.'s study [13] included 138 patients with AS and 140 healthy controls for TLR4 Asp299Gly and Thr399Ile polymorphisms. Their study showed no significant differences in allele or genotype frequencies between controls and AS patients. They pointed out that TLR4 signalling pathway seem not to be genetically determined by

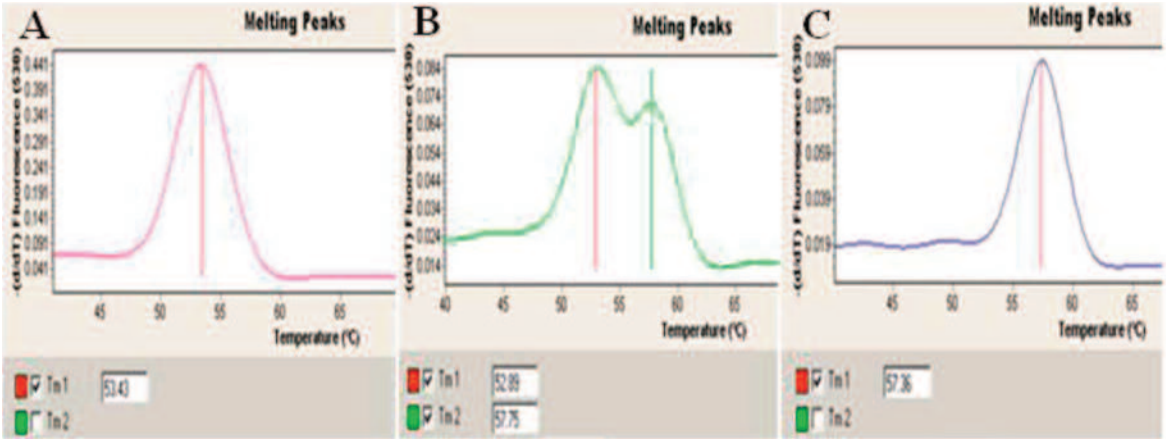

Figure1. Homozygous wild type alleles (A896A) (A); Heterozygous mutated alleles (A896G) (B); Homozygous mutated alleles (G8946G) (C).

these two common polymorphisms. Pointon *et al*. [14] assesed 522 United Kingdom probands and 516 sex-matched controls. No association was found about TLR4 with AS. In a meta-analysis, Xu *et al.* [15] reviewed all of the studies discussing the relationship between TLR4 D299G/T399I and rheumatoid arthritis/AS. Their study suggested that TLR4 D299G/T399I polymorphisms are not associated with rheumatoid arthritis/AS. Na *et al*.'s study [16] included 200 Korean AS patients and 197 healthy controls. All subjects were genotyped for two functional single necleotide polymorphisms (SNPs) in the TLR4 gene: Asp299Gly (A/G) and Thr399Ile (C/T). They indicated that TLR4 gene polymorphisms cannot be regarded as major contributors to AS in Korean population. Adam *et al.* [17] compared the frequency of two TLR4 mutations (Asp299Gly and Thr399Ile) in 193 AS patients and 125 HLA-B27 healthy controls. The results of their study showed that 29/193 (15%) patients with AS had a polymorphism in the Asp299 site compared with 18/125 (14.4%) healthy HLA-B27 controls and 29/184 (15.8%) patients with AS had a polymorphism in the Thr399Ile site compared with 19/113 (16.8%) HLA-B27 controls. No significant difference was found in allele frequency in AS and healthy HLA-B27 controls. Van der Paardt *et al.* [18] investigated the distribution of the CD14C-260T and TLR4 A896G polymorphisms in 113 unrelated white Dutch AS patients and 170 healthy controls. The results of their study showed no significant differences between patients and controls in the frequencies of the TLR4 896G allele.

Yang *et al.* [19] investigated TLR4, TNF-α, IL-12 and soluble tumour necrosis factor-related apoptosis-inducing ligand (sTRAIL) in 60 patients with AS (38 HLA-B27 positive and 22 HLA-B27 negative), 20 patients with rheumatoid arthritis and 30 patients with healthy volunteers. Their results showed that TLR4 levels were significantly higher in AS patients than healthy controls. They reported that TLR4 plays an important role in the pathogenesis of AS, as independent of HLA-B27. De Rycke *et al*. [20] investigated TLR2 and TLR4 gene polymorphisms in 23 patients with spondylarthropathy, 15 patients with rheumatoid arthritis and 18 patients with osteoarthritis. As a result of their study TLR4 expression was increased in patients with spondylarthropathy. They suggested that inflammation in spondylarthropathy is characterized by increased TLR4 expression and is reduced by tumour necrosis factor-α (TNF-α) blockade. Snelgrove *et al*.'s study [21] included 101 AS patients and 100 healthy controls for 2 variants in the TLR4 gene: Asp299Gly (A/G polymorphism) and Thr399Ile (C/T polymorphism). As a result of their study, the frequency for Asp299Gly variant (G) was significantly higher in AS cases compared to controls (7.5% vs 2.6%, respectively; OR: 3.10, $p = 0.037$). The minor allele frequency for the Thr399Ile variant (T) for cases and controls was 7.4% vs 3.0% ($p = 0.071$). They suggested that TLR4 has a minor risk factor for AS.

Assassi *et al*.'s study [22] is the only study about the relationship between TLR4 gene polymorphism and disease activity in AS patients. Their study included 16 patients with AS, 14 matched controls, 74 patients with systemic sclerosis, 21 matched controls and 17 patients with systemic lupus erythematosus. Also these genes were investigated in 27 patients with AS (before and after anti TNF-α treatment) and 27 matched controls. Disease activity of the AS patients

was evaluated with BASDAI score and CRP were measured from all AS patients. As a result of their study over expression of TLR4 and TLR5 was found in AS patients in comparison to controls ($p = 0.012$ and $p = 0.006$, respectively). TLR4 and TLR5 were significantly upregulated among the AS patients before anti TNF-α treatment ($p = 0.007$ and $p = 0.012$, respectively) and were decreased significantly after anti TNF-α treatment ($p = 0.002$ and $p = 0.025$, respectively). CRP levels were correlated with TLR4 and TLR5 levels ($p = 0.015$, $p = 0.001$, respectively). BASDAI scores did not correlate with TLR4 and TLR5 levels. Their study supported the importance of TLR subtypes in the pathogenesis of AS.

As a result in some studies no significant differences was found in TLR4 gene polymorphism between controls and AS patients [12-17]. But we confirmed the overexpression of TLR4 gene polymorphism among patients with AS, similar to some previous studies [18-21]. This can be due to the activation of this TLR4 cytokine pathway, TNF-α and other proinflammatory cytokines are expressed. These proinflammatory cytokines are expressed also in AS [4]. We didn't find any association between TLR4 gene polymorphism and ESR, CRP levels and BASDAI score. In Assassi *et al*.'s study [22], CRP levels were correlated with TLR4 and TLR5 levels but BASDAI scores did not correlate with TLR4 and TLR5 levels. Our study differs in this respect.

The Limitations of the Study

The limitations of our study were that we evaluated the frequency of TLR4 gene polymorphism and its relationship between TLR4 gene polymorphism and disease activity in a small number of patients. As a result of this limitation more studies with more patients are warranted to elucidate the frequency of TLR4 gene polymorphism in AS and its relationship between disease activity.

Conclusions

Our study revealed that TLR4 pathway is important in AS. We confirmed the overexpression of TLR4 gene among patients with AS. But, this polymorphism was not associated with disease activity, ESR, CRP levels.

Authorship declaration

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Sensitivity of localization studies performed by various radiologists in the evaluation of parathyroid lesions

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ABSTRACT

Objective. Preoperative imaging studies are commonly used in the diagnosis of primary hyperparathyroidism to increase the success rate of surgery. In the present study, we aimed to correlate surgical outcomes with the sensitivity of localization studies that were performed by various radiologists. ***Methods.*** One hundred eighty-nine patients with preoperative diagnosis primary hyperparathyroidism were included. A total of 174 patients in whom hypercalcemia had been cured by parathyroidectomy, were evaluated retrospectively. In total, 184 lesions were excised from these 174 patients. Ultrasonography (USG) and technetium-99m-methoxy isobutyl isonitrile (99mTc-MIBI) imaging yielded correctly localized lesion in 74 and 108 patients, respectively. ***Results.*** The specificity of USG and 99mTc-MIBI imaging were similar (95.9% and 95.0%, respectively). However, the sensitivity was not satisfactory (45.9% for USG, 62.4% for 99mTc-MIBI). The gland size was not significant for the rate of lesion detection by 99mTc-MIBI scan or USG. Significant differences were not observed between the preoperative serum parathormone, serum calcium or 24-hour urine calcium excretion levels and the success rate of localization with either USG or 99mTc-MIBI. ***Conclusions.*** Radiologist experience in ultrasonographic parathyroid imaging was found to affect sensitivity. Therefore, surgeons, radiologists and endocrinologists that perform ultrasonographic evaluation should have extensive experience.

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Keywords: Primary hyperparathyroidism, parathyroidectomy, localization studies, ultrasonography, technetium-99m-methoxy isobutyl isonitrile

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Introduction

Primary hyperparathyroidism is characterized by hypercalcemic clinical manifestations that emerge as a result of excess parathyroid hormon (PTH) secretion from the parathyroid gland. Its prevalence increases with age and reaches to 2% in the population that is between 50 and 60 years of age [1, 2]. Most patients are asymptomatic. The increase in routine measurement of serum calcium levels has increased early diagnosis and estimates of primary hyperparathyroidism prevalence [3]. Solitary parathyroid adenoma is the most common cause of primary hyperparathyroidism (80-85%), followed by diffuse or nodular hyperplasia (10-15%), multiple parathyroid adenoma (2-5%), and carcinoma (<1%) [4]. Depending on its severity, hypercalcemia can lead to several complications, particularly in the renal, gastrointestinal, cardiovascular, neurologic and musculoskeletal systems. Surgery is the only curative therapy that can relieve short- and long-term complications related to hypercalcemia [5]. Preoperative localization studies are important for enhancing surgical success. However, they do not involve diagnostic criteria because the methods employed yield both false negative and false positive results.

Ultrasonography (USG) and technetium-99m-methoxy isobutyl isonitrile (99mTc-MIBI) imaging are primary imaging techniques in preoperative evaluation. USG is an inexpensive, sensitive and specific modality that does not expose the patient to radiation. Although the sensitivity and specificity of 99mTc-MIBI imaging is higher than those of USG, it involves low levels of radiation exposure. Unlike USG, 99mTc-MIBI imaging is not operator dependent [6]. The sensitivities of these modalities vary among centers, with a mean preoperative USG sensitivity of 76% (48-98%) for USG and of 79% (61-100%) for 99mTc-MIBI imaging [7]. However, the sensitivity of USG and 99mTc-MIBI imaging decreases to 34.8% and 44.4%, respectively, in multiglandular disease and to 16.2% and 29.9%, respectively, in double parathyroid adenomas. These decreases in multiglandular disease and double adenoma may be related to small gland sizes or insufficient investigation by the radiologist following the detection of an abnormal parathyroid gland [7, 8]. Gland size and volume are the most powerful independent factors that affect the success of localization methods. Accordingly, a correlation between both preoperatively high serum PTH and calcium levels and the success of localization method has been reported [9]. The presence of ectopic pathological gland does not affect the sensitivity of 99mTc-MIBI imaging but negatively affects USG sensitivity [10, 11]. In addition to all these factors, radiologist experience in imaging the parathyroid gland plays an important role [7].

In the present study, we aimed to investigate the factors influencing the sensitivity of preoperative localization methods and compare the results to the literatüre.

Methods

In our study, we included patients who were diagnosed with primary hyperparathyroidism between 2005 and 2016 and operated on in our center. This study was approved by the Institutional Review Board of the Uludağ University. Patient data were collected from electronic files. The preoperative presenting complaints and biochemical results of 189 patients were analyzed. Curative result rates were evaluated with serum PTH and calcium levels on postoperative day 1 and at 3 and 12 months during follow-up. Among those patients whose hypercalcemia was treated after surgery, the preoperative sensitivity, specificity, true positivity and true negativity of USG and 99mTc-MIBI measurements by different radiologist were assessed. The correlations between each of sensitivity rate and specificity rate in imaging studies and macroscopic measurement of postoperatively resected lesions in pathological assessment were evaluated. In addition, the correlation between imaging method success and preoperative biochemical parameters were analyzed.

Statistical Analysis

Statistical analysis was performed using the computer software SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). The chi-square test was used for the comparison of categorical data, whereas the Mann-Whitney U test or Student t-test were used for the comparison of numerical data as appropriate. Demographic characteristics and preoperative frequency data were expressed as the means and standard deviations (SD) and percent (%) where appropriate. The sensitivity and specificity of the preoperative examinations were calculated manually with the number of true and false scores according to

the postoperative results. A $p < 0.05$ was considered statistically significant.

Results

Among the 189 patients, 162 (85.7%) were female and 27 (14.3%) were male. The mean age was 53.2 ± 12.2 years. There were 63 (33.3%) symptomatic patients diagnosed as primary hyperparathyroidism while they were investigated for seconder osteoporosis (13.2%) and urolithiasis (9.5%). Three patients had multiple endocrine neoplasia (MEN) syndrome. Twelve (6.3%) patients were administered bisphosphonate therapy because of preoperative severe hypercalcemia. The mean preoperative serum calcium level was 11.5 ± 1.0 mg/dl, and the mean preoperative serum PTH level was 414 ± 527 pg/ml. Table 1 shows the patients' demographic and preoperative biochemical data.

We performed a total of 199 surgeries because of persistent hyperparathyroidism, 11 patients were operated on twice, and 1 patient was operated on triple. Two of the patients had been referred to our hospital after noncurative first surgery at their centers. We obtained a curative result in 162 (86.6%) of 187 hypercalcemic patients whose first operation was at our center. Among the 11 patients who underwent a second surgery, 72.7% had a curative result. Considering all the operations, 92.0% of 189 patients were treated with surgery (Table 2). In histopathological evaluation, solitary adenoma was observed in 178 patients, double adenoma was observed in 9 patients, and parathyroid carcinoma was observed in 1 patient. One patient underwent 3.5-gland parathyroidectomy because of multiple gland hyperplasia (Table 3). In the other MEN patients it is observed that dominant adenoma was resected on the first operation and other glands were not checked during surgery. So these patients had to be reoperated. The sensitivity of the imaging modalities was calculated by comparing the results of the preoperative localization studies with the resected side in patients with curative surgery. Among the 174 patients who underwent curative surgery, USG had been performed with 155 patients, and 99mTc-MIBI imaging had been performed with 167 patients. USG localized lesions correctly in 74 (47.7%) patients, whereas 99mTc-MIBI imaging did so in 108 (64.4%) patients. The sensitivity rate was 45.9% for USG and 62.4% for 99mTc-MIBI imaging. The specificity rate was 95.9% for USG and 95.0% for 99mTc-MIBI imaging (Table 4).

In our study, patient gender did not affect

Table 1. Demographic characteristic of patients and preoperative laboratory findings

	Patients (n = 189)
Sex	
Male	27
Female	162
Age (years)	53.2 ± 12.2 (19 - 88)
Asymptomatic	126
MEN	3
Pre-op calcium (RR: 8.4-10.2 mg/dL)	11.5 ± 1.0 (10.2 - 18.5)
Pre-op phosphorus (RR: 2.4-4.4 mg/dL)	2.4 ± 0.5 (0.8 - 4.1)
Pre-op creatinine (RR: 0.7-1.1 mg/dL)	0.74 ± 0.20 (0.48 – 1.8)
Pre-op PTH (RR: 15-68.3 pg/mL)	414 ± 527 (71 - 4262)
Pre-op vitamin D (RR: >30ng/L)	14.7 ± 8.9 (2.1 – 54)
Pre-op 24 h U Ca (RR: <400 mg/day)	388 ± 253 (60 - 2062)
Associated thyroid disease	63

Data were expressed as the means ± standard deviations (range) and number. MEN = multiple endocrine neoplasia, Pre-op = preoperative, PTH = parathormone, 24 H U ca = 24-hour urine calcium excretion

Table 2. Results of operation

	Cure rates
1st operation (n = 187)	165 (86.6%)
2nd operation (n = 11)	8 (72.7%)
3rd operation (n = 1)	1 (100%)

Data were expressed as number (percent).

Table 3. Histopathological features

	Patients (n = 155)
Single adenoma	178
Double adenoma	9
Single gland hyperplasia	0
Multi-gland hyperplasia	1
Carcinoma	1
Non-pathological gland	9

Table 4. Results of preoperative localization studies

	USG (n = 155)	^{99m}Tc-MIBI (n = 167)
True positive	74	108
False positive	6	8
False negative	87	65
True negative	143	153
Sensitivity (%)	45.9	62.4
Specificity (%9	95.9	95.0

USG = ultrasonography, ^{99m}Tc-MIBI = technetium-99m-methoxy isobutyl isonitrile

Table 5. Comparisons of patients with positive and negative USG or MIBI results

	USG			^{99m}Tc-MIBI		
	positive	*negative*	*p-value*	*positive*	*negative*	*p-value*
Sex (M/F)	12/61	14/87	NS	18/87	8/61	NS
Age	51.9 ± 12.3	53.4 ± 12.2	NS	52.6 ± 12.0	53.0 ± 12.6	NS
PTH	436.6 ± 448.2	342.4 ± 375.9	NS	391 ± 375	367 ± 458	NS
Ca	11.6 ± 1.2	11.3 ± 1.0	NS	11.5 ± 1.18	11.2 ± 0.92	NS
P	2.39 ± 0.66	2.54 ± 0.53	NS	2.41 ± 0.59	2.57 ± 0.58	NS
24-h U Ca	386 ± 196	350 ± 302	NS	387 ± 217	333 ± 320	NS
Size (mm)	22.3 ± 11.1	20.3 ± 9.3	NS	22.1 ± 10.6	19.7 ± 9.1	NS

Data were expressed as the means ± standard deviations and number. USG = ultrasonography, ^{99m}Tc-MIBI = technetium-99m-methoxy isobutyl isonitrile, PTH = parathyroid hormone, Ca = calcium, P = phosphorus, 24-h Ca = 24-hour urine calcium excretion, NS = non-significant ($p > 0.05$)

sensitivity ($p>0.05$). There was no significant association between gender and imaging modality success ($p>0.05$). Analysis of the correlations between preoperative biochemical parameters and imaging modality success revealed no significant association between preoperative serum calcium, PTH, phosphorus or 24-hour urine calcium excretion levels and successful localization. Parathyroid gland size did not affect the success of the imaging modality (Table 5).

Discussion

Parathyroid adenoma and hyperplasia are the most common causes of primary hyperparathyroidism; parathyroid carcinomas are very rare [3]. Primary hyperparathyroidism due to adenoma is common in older women [1, 2]. The surgical resection of the adenoma is the only curative therapy in the patients with primary hyperparathyroidism. Parathyroid adenomas are typically small in size and can go undetected by preoperative imaging methods in some cases [12, 13]. The main therapeutic approach is to detect and resect the adenoma during operation with neck exploration even if the adenoma is not detected preoperatively. The preoperative localization of the adenoma and the surgeon's experience are crucial to the success of surgery [7].

Among the 189 subjects in our study, 162 were female (F/M: 6/1). Three patients had hyperplasia, and one patient had parathyroid carcinoma; these findings are consistent with the literature. In the literature, the

curative operation rate is greater than 95% in experienced centers. In our study, this rate was 92.0%, which is slightly lower than that of experienced centers. It was thought that our success rate should increase with experience. The slightly lower rate observed in the present study may also be due to the exclusion of patients who had no postoperative follow-up at our center. Patients that were operated on and cured may have discontinued outpatient follow-up postoperatively.

The preoperative detection rate of parathyroid adenoma varies among imaging methods and studies. Reviews have indicated that USG and 99mTc-MIBI imaging are the most sensitive methods [5]. The sensitivity and specificity of USG are reported to be 56-100% and 50-99%, respectively [12]. For 99mTc-MIBI imaging, they are 56-100% and 83-99%, respectively [12]. In our study, the sensitivity and specificity of 99mTc-MIBI imaging are consistent with those reported in the literature. The sensitivity of 99mTc-MIBI imaging may reach very high levels if used carefully, and the specificity of this method is high. The positive predictive value obtained for USG in the present study is consistent with the literature, and the false positive rate was low. However, the sensitivity of USG was low, and the false negative rate for USG was high.

It has been reported that adenomas are preoperatively detected more with USG than 99mTc-MIBI, especially when USG is performed by experienced hands [13]. The rate of curative surgery is high in patients with adenomas that were localized preoperatively. In addition, in these patients, the need for wide neck exploration during surgery and the rate of complications decrease. However, the resection of adenomas via minimal surgical intervention with local anesthesia is reported to be possible [14].

Surgery is the only curative therapy, and bilateral neck exploration is considered the gold standard surgical approach. All the parathyroid glands can be evaluated by this procedure, and the rate of curative surgery is approximately 95% [7, 15]. The rate of curative surgery decreases for adenomas that cannot be localized with preoperative imaging methods [16]. Therefore, there is a correlation between surgical success and the sensitivity of localization studies. In our study, for USG, the true positive rate was 47.7%, the false negative rate was 56.1% and sensitivity was 62.4%; for 99mTc-MIBI imaging, the corresponding values were 64.6%, 38.9% and 62.4%. Although 99mTc-MIBI imaging was superior to USG in our study, some studies have reported that USG is superior to 99mTc-MIBI imaging [17]. USG, performed by experienced radiologist, may be superior to 99mTc-MIBI [17]. Sensitivity increases when the two modalities are used together. In addition, radiologist experience is an important factor affecting sensitivity [18, 20]. The lower sensitivity of USG and 99mTc-MIBI observed in our study relative to their sensitivity in previous studies might reflect the fact that the imaging studies were performed by different radiologists not all of whom are experienced in parathyroid imaging.

In the literature, gland size and volume are reported to be most powerful independent factors influencing successful localization [7]. It is known that small lesions are more difficult to localize than large lesions in USG. However, the difficulty in detecting small lesions in 99mTc-MIBI imaging might be explained by lower intracellular radiotracer uptake in small lesions [17]. In our study, size did not significantly affect the USG and 99mTc-MIBI imaging results.

Some studies have reported that preoperative serum PTH and calcium level were both higher in patients whose imaging study results are positive [9]. Vassy *et al.* [20] found that PTH and calcium levels did not affect 99mTc-MIBI imaging results in 37 patients with primary hyperparathyroidism. We did not observe a significant correlation between any of preoperative serum PTH, serum calcium, and 24-hour urine calcium excretion levels and successful localization in our study.

There is concomitant thyroid pathology in 20-30% of patients with primary hyperparathyroidism [21]. The presence of thyroid pathology decreases the sensitivity of both USG and 99mTc-MIBI imaging [22-24]. In 99mTc-MIBI imaging, this decrease in sensitivity might be associated with a delay in wash-out in multinodular goiter and Hashimoto's thyroiditis, whereas in USG, this decrease might be associated with the limitation of sonographic evaluation of adenoma because of a multinodular thyroid gland. However, it has been reported that benign and malign thyroid diseases might cause false positive results [22-24]. In our study, 33% of patients had concomitant thyroid disease. There was no significant difference in sensitivity of either USG or 99mTc-MIBI imaging between patients with thyroid nodule and without thyroid nodule.

Conclusions

In conclusion, the treatment of primary hyperparathyroidism is surgical resection in parathyroid adenomas and surgical resection of almost the entire parathyroid gland, leaving minimal residual tissue, in parathyroid hyperplasia. Surgery should be performed by operators specialized in parathyroid surgery. Preoperative localization of a lesion is not an indication for surgery, but it increases the rate of curative surgery and decreases surgical complications. Therefore, it is important to carefully evaluate patients using a multidisciplinary approach involving a surgeon, an endocrinologist and a radiologist.

Authorship declaration

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Pedunculated liver hemangioma mimicking stomach neoplasm

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ABSTRACT

Hepatic hemangiomas are the most frequently seen benign liver mass. However, exophytic hepatic hemangiomas, particularly pedunculated ones are very rare. They have various appearances that make the diagnosis difficult. We report a pedunculated hemangioma case which was misdiagnosed as gastrointestinal stromal tumor on computerized tomography scan. Magnetic resonance imaging revealed that the tumor was a hepatic hemangioma with a long peduncle originating from the right edge of the liver, extending into perigastric area. Hepatic hemangioma with a long peduncle can be mistaken with other abdominal masses. Remembering this entity and knowing its imaging characteristics are the keys to diagnosis.

Eur Res J 2018;4(2):118-121

Keywords: Pedunculated hemangioma, liver, gastric mass

Introduction

Hepatic hemangiomas are the most frequently seen benign liver masses (7-20%), which are seen more often in woman. They are generally single and located frequently in right lobe especially posterior segment and subcapsular area [1]. They are usually less than 3 cm in diameter, larger ones (> 4 cm) are named as giant hemangioma [2]. Hepatic hemangiomas are asymptomatic and discovered incidentally. Larger lesions may cause symptoms, like a palpable abdominal mass, pain, hemorrhage, jaundice, nausea/vomiting. Exophytic hepatic hemangiomas, particularly pedunculated ones are very rare [3]. Atypical hemangiomas like pedunculated ones have various appearances that make diagnosis difficult.

Case Presentation

A 70-year-old man with an incidentally found intra-abdominal mass located adjacent to greater curvature of stomach on abdominal ultrasound was referred to radiology department for abdomino-pelvic computerized tomography scan. He was asymptomatic despite the mass and had no family history of abdominal malignancy. On physical examination, there were no abnormal findings. Portal venous phase computerized tomographic images revealed a 22 × 26 mm exophytic mass located adjacent to greater curvature of the stomach (Figures 1a and 1b), mass was considered as gastrointestinal stromal tumor. Endoscopic examination and magnetic resonance imaging was recommended. In addition, there was another 20 × 15 mm mass located at segment 6 of

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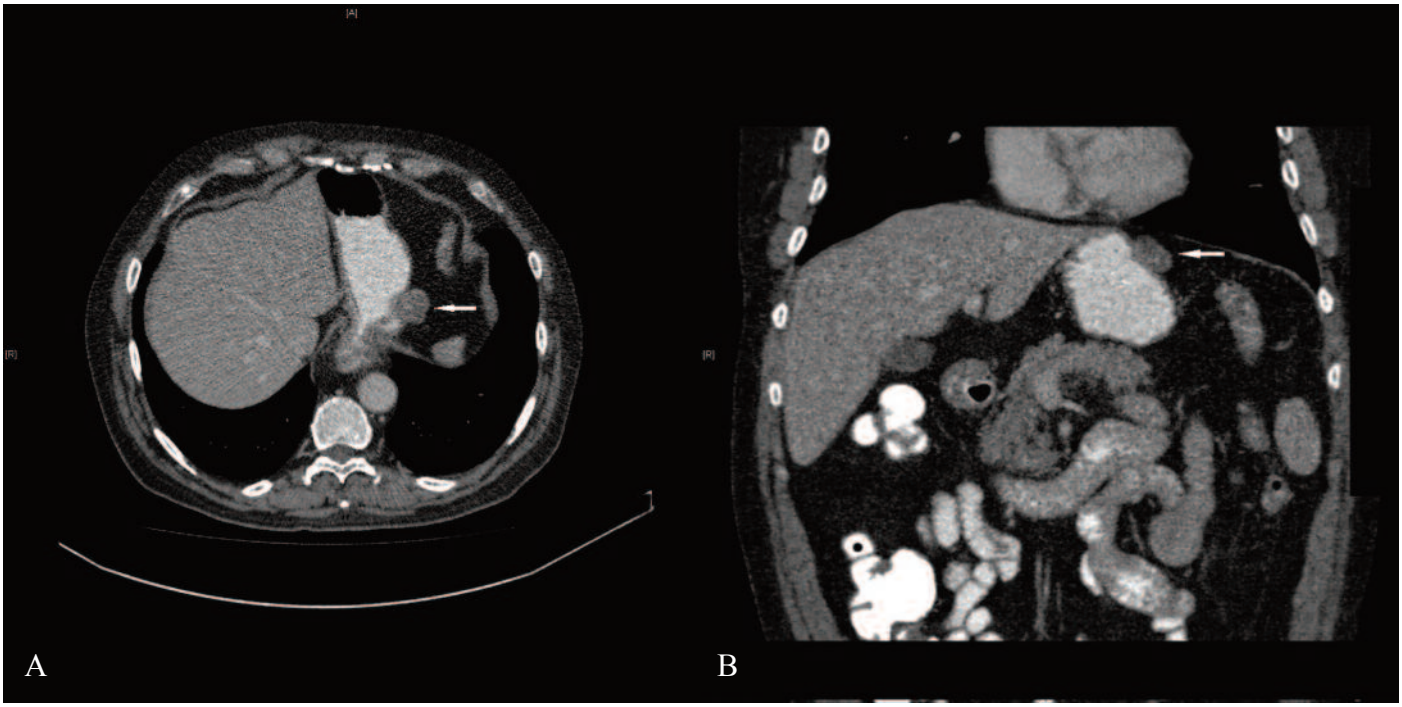

Figure 1. Axial (a) and coronal reformat (b) contrast enhanced CT images showing an exophytic , non enhanced, mass (arrow) located at great curvature of stomach.

liver, with peripheral enhancement. This lesion was diagnosed as hemangioma.

Endoscopic findings were normal. On magnetic resonance imaging, the lesion at segment 6 of liver had peripheral nodular discontinuous enhancement which progressed centripetally on delayed images, consistent with hemangioma. The other lesion, which was considered to be gastrointestinal stromal tumor according to computerized tomography, was originating from left liver lobe and extending exophyticly into perigastric area. It had the same hemodynamic characteristics with the lesion at segment 6 and was seen to be connected to left liver lobe by a peduncle, thus it was described as pedunculated liver hemangioma (Figures 2a, 2b, 2c, and 2d).

Discussion

Hemangiomas are the most common benign tumors of the liver, and the diagnosis of typical hemangioma is easy with the help of various imaging techniques, like ultrasound, computerized tomography or magnetic resonance imaging. However pedunculated hepatic hemangioma is very rare. As far as we know there is 18 reported cases in English literature. Pedunculated hemangioma is known to mimic other abdominal masses, like gastric tumor or other pedunculated hepatic tumors, such as hepatocellular carcinoma, mesenchymal hamartoma, focal nodular hyperplasia, or adenoma. Diagnosis of pedunculated hepatic hemangioma might be difficult as defining the origin of a pedunculated mass is generally problematic [4].

Ultrasound, computerized tomography, magnetic resonance imaging, radionuclide scintigraphy, and angiography are diagnostic methods to identify pedunculated hepatic hemangiomas. Generally, it is difficult to show the origin of the lesion on ultrasound. If the lesion is attached to the liver by a thin pedicle, the peduncle can be nearly undetectable by imaging methods. Multiplane reconstruction computerized tomographic images and coronal or sagittal magnetic resonance imaging may be helpful in diagnosis. On computerized tomography and magnetic resonance imaging, the diagnosis is made by the demonstration of the typical enhancement patterns / signal intensities on both T1- and T2-weighted images [4, 5].

In current case, computerized tomography failed to diagnose the lesion because it was not a dynamic examination. Thus, the typical enhancement pattern for hemangioma could not be revealed by computerized tomography. In addition, the localization of the lesion is atypical for a hepatic mass and the peduncle of the lesion was clearer in magnetic resonance imaging.

Hemangiomas are mostly asymptomatic, but

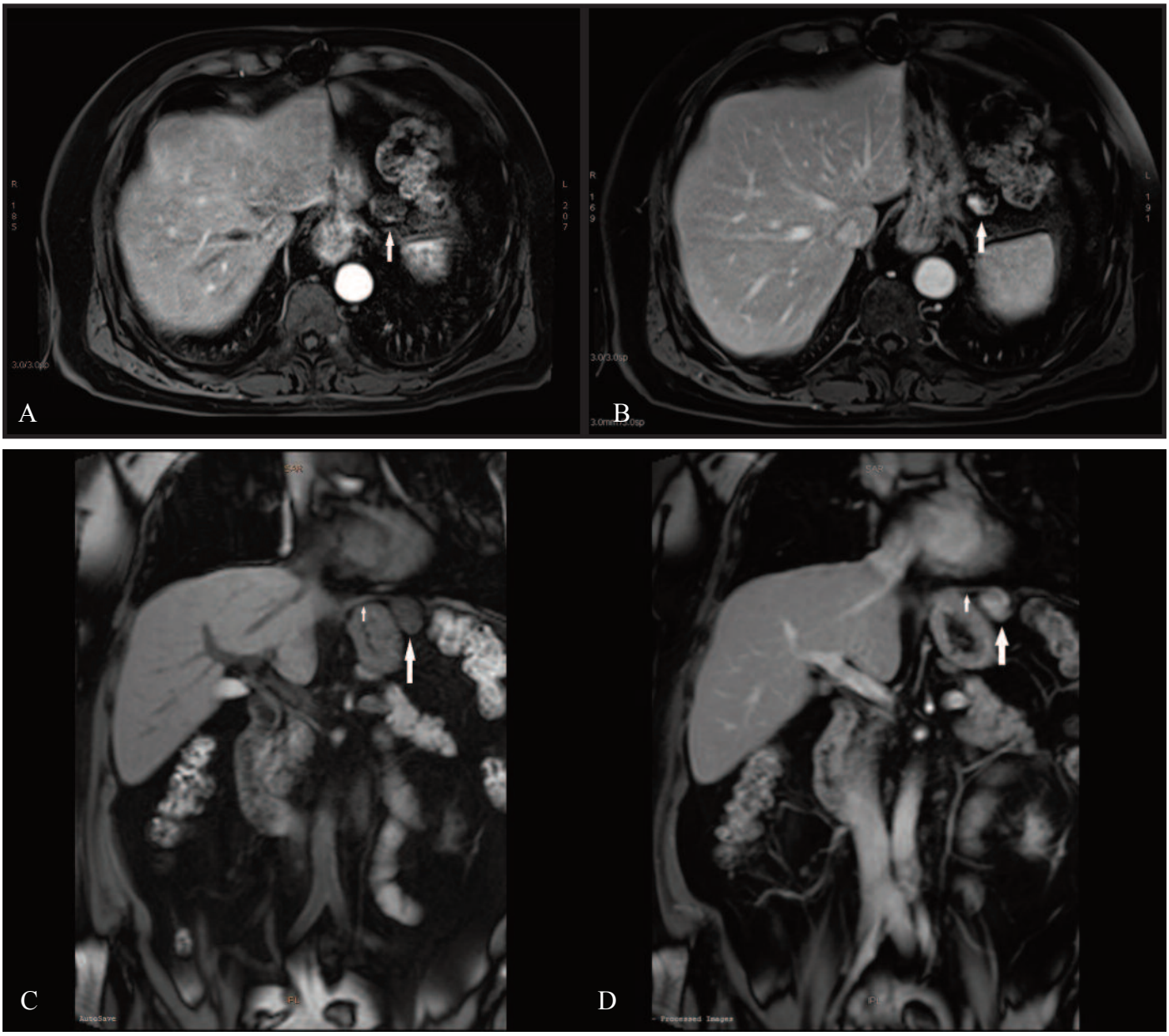

Figure 2. Early arterial (a), portal venous (b) and delayed, contrast enhanced T1 weighted images showing the pedunculated hemangioma (arrow) which has peripheral nodular discontinuous enhancement, progressed centripetally on delayed images. On coronal reformatted non-contrast enhanced (c) and contrast enhanced (d) images, the peduncle of the hemangioma can be seen more easily (peduncle: small arrow, hemangioma: long arrow).

larger ones may produce certain symptoms, including an abdominal mass, pain, nausea, vomiting, jaundice, hemorrhage and sometimes rupture. There is no need for treatment for the majority of hemangiomas, except the ones which present with a palpable mass, pain or complications, as consumptive coagulopathy and rupture can be treated with surgery. Pedunculated hepatic hemangiomas can be complicated by subacute torsion and infarction, and complicated ones must be resected immediately [3, 5].

Conclusion

Pedunculated hepatic hemangiomas are uncommon and rare benign liver masses. They show similar imaging features with typical hemangiomas. They should be kept in mind in the differential diagnosis of an upper abdominal mass. Imaging modalities including ultrasound, computerized tomography and magnetic resonance imaging are all capable to diagnose these tumors. Remembering the imaging characteristics and identifying the peduncle are crucial in achieving correct diagnose and avoiding unnecessary diagnostic or therapeutic interventions.

Informed consent

Written informed consent was obtained from the patient for the publication of this case report.

Conflict of interest

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contributions

Study concept and design: EF, SA; Acquisition of data: EF, SA; Analysis and interpretation of data: EF, SA; Drafting of the manuscript: EE, SA; Critical revision of the manuscript for important intellectual content: EE, PNK; and Study supervision: PNK.

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Borderline Brenner tumor with a concomitant mucinous tumor of the ovary: a case report

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ABSTRACT

Brenner tumors (BTs) are rare ovarian tumors which are a part of the epithelial stromal tumor group of ovarian neoplasms. Most of the BTs are benign and usually asymptomatic. BTs stand for the 1.4-2.5% of ovarian tumors. Of the BTs, not more than 2% exhibit characteristics of borderline (or malignant) BTs. Borderline BTs are usually bigger than benign BTs and individuals typically represent with symptoms related with a unilateral ovarian mass. BTs are often related with mucinous cystadenomas or seldom cystadenocarcinomas. We present a case of borderline BT associated with a concomitant mucinous ovarian tumor.

Eur Res J 2018;4(2):122-125

Keywords: Brenner tumor, borderline Brenner tumor, ovarian tumor

Introduction

Brenner tumors (BTs) are defined by Fritz Brenner as oophoroma folliculare ovarii in 1907, and comprise 5% of benign tumors of the ovary [1]. They are comprised of layers of urothelium-like epithelial cells, that are encircled by condensed fibrous stroma and are thought to rise from superficial epithelium of the ovary that suffers transitional metaplasia. BTs comprise nearly 1.4-2.5% of tumors of the ovary [2]. World Health Organization categorized BTs into 3 groups: malignant, borderline and benign. The benign ones are the most frequent, representing about 95%, the borderline represent about 5%, and the malignant ones less than 1% [3]. Borderline BTs are epithelial tumors that appear as cellular islands or irregular epithelial masses that predominate in the cystic areas, differentiated by a dense conjunctive tissue at the periphery. BTs are more frequently related with cystadenomas or infrequently cystadenocarcinomas that are thought to be a kind of BT's metaplastic variation [4]. We present a case of borderline BT associated with a concomitant mucinous ovarian tumor.

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Case Presentation

A 54-year-old postmenopausal G2P2 female patient applied to our clinic with the complaints of abdominal distension, and inguinal pain which she noted two months ago. Medical history of the patient was unremarkable. Pelvic computed tomography demonstrated a multicystic lesion measuring 26×23×12 cm with a solid component, freely floating fluid around the mass and in the pelvic area (ascites?), millimetric soft tissue densities in the mesenteric adipose tissue at the left side of the mass, and blurred/thickened omentum lateral to the mass (peritoneal carcinomatosis?) (Figure 1). Preoperative tumor marker CA-125 was measured as 33 kU/L.

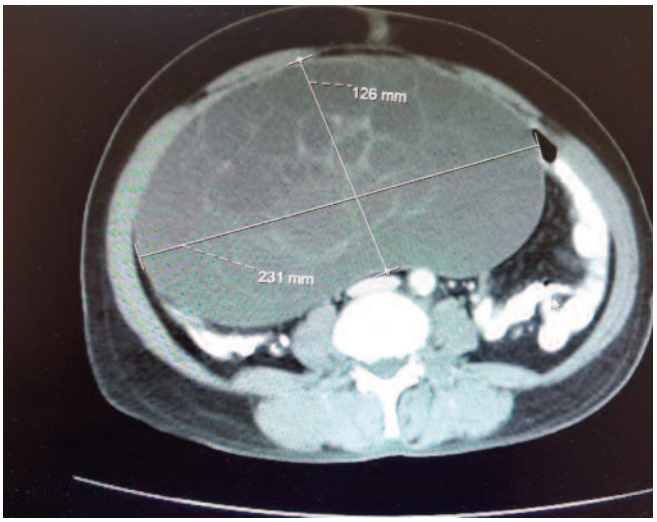

Figure 1. Pelvic computed tomography showing a multicystic lesion measuring 26×23×12 cm with a solid component

Midline laparotomy was performed. Abdominal exploration revealed a 30 cm-bulky tumor originating from the left ovary (Figure 2). Contralateral ovary was apparently normal. The result of frozen section performed during surgery was interpreted as a "malign" lesion. The patient underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, bilateral pelvic paraaortic lymphadenectomy, infracolic omentectomy, appendectomy, and multiple peritoneal biopsies were also performed.

Postoperative pathology was interpreted as "borderline BT originating from the left ovary and concomitant mucinous ovarian tumor". Histopathological analysis of all other samples were reported as normal. On immunohistochemical staining, p-63-positive, while WT-1 negative nuclei were observed (Figure 3).

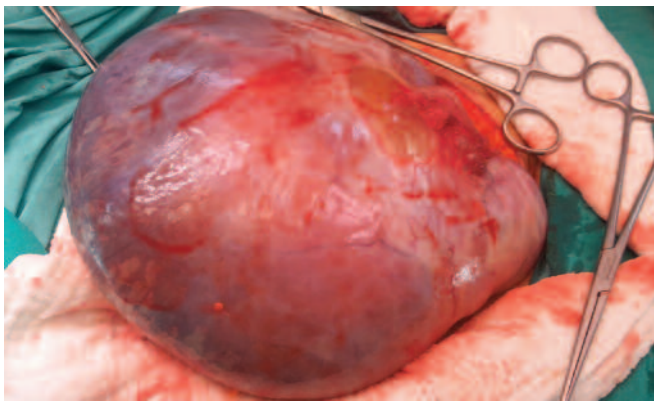

Figure 2. A 30 cm-bulky tumor originating from the left ovary.

The patient who did not experience any complication during postoperative period was discharged. Follow-up of this stage 1A patient was planned. From January 2017, up to now she hasn't experience any disease recurrence.

Discussion

Benign BTs are seen during the 4th or 5th decade, while borderline and malignant BTs appear approximately 10 years later. Individuals commonly pose with symptoms related with an ovarian mass at one side. Borderline BTs are characteristically bigger than benign BTs, with an average size of 16 to 20 cm. Abdominal exploration of the present case revealed a 30 cm-bulky tumor originating from the left ovary. Contralateral ovary was apparently normal.

p-63 immunoexpression has been confirmed to be a valuable sign in normal uroepithelium and upper urinary tract. Earlier morphological reports have revealed that BTs display urothelial differentiation [5]. Liao *et al.* [6] revealed that p-63 is shown in all benign and borderline BTs, and also revealed that most malignant BTs had deficiency of p-63 immunoexpression, implies that p-63 may take role in ovarian BTs pathogenesis. In the present report, the patient showed p-63-positivity.

Typical definition of an ovarian BT by computed tomography and magnetic resonance imaging is broad and vague calcification in a solid element in a multilocular cystic lesion [7]. On computed tomography, the benign component contains intense calcifications; whereas on magnetic resonance imaging, benign component shows very low intensity on T2-weighted images, and malignant component

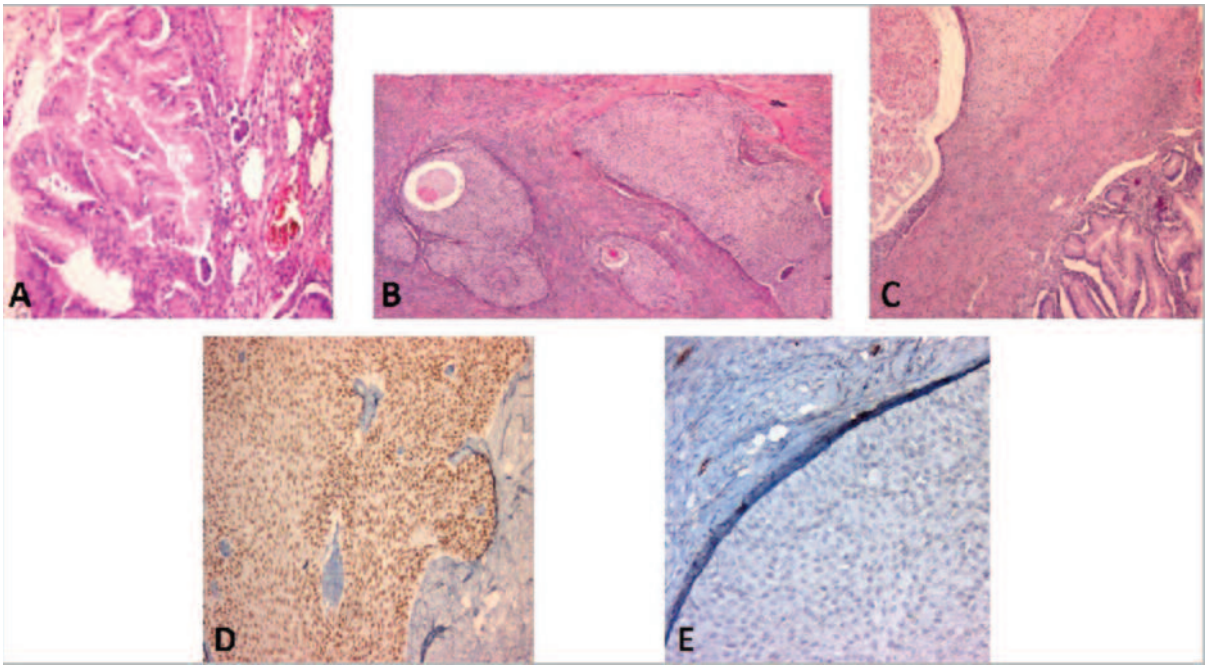

Figure 3. A. Mucinous boderline tumor with microinvasive carcinoma (H&E stain 10×). B. Atypical proliferative (borderline) Brenner tumor. Large nest of proliferating transitional-type epithelium in center of field with extensive stratification (H&E stain 5×). C. Mucinous boderline tumor and Brenner borderline tumor. (H&E stain 5×). D. Atypical proliferative (borderline) Brenner tumor. Immunohistochemical stain for p63 is positive (10×). E. Atypical proliferative (borderline) Brenner tumor. Immunohistochemical stain for WT-1 is negative (20×).

shows high intensity. In the present report, pelvic computed tomography demonstrated a multicystic lesion with a solid component, freely floating fluid around the mass and in the pelvic area.

The borderline BT of the ovary continually had a pleasant prognosis and show a benign clinical progression after oophorectomy. The greater part of BTs are benign; thus, timely and precise detection of a malignant tumor would confirm that the individual obtains the exact surgery required [8].

Brenner tumors are frequently related with cystadenomas or infrequently with cystadenocarcinomas [4]. It is noteworthy that nearly ¼ of mucinous ovarian tumors, a small BT element is detected [9]. The co-existence of germ-cell tumors such as mature cystic teratoma, detected in our report as well, though infrequent is deliberated by individual authors as sign of a germ cell origin of the BTs [4, 9]. Most BTs are benign. Strict measures similarly present for the categorization of borderline BTs. A new classification of borderline BTs is suggested: grade 1 (borderline, not otherwise specified) and grade 2-3 (borderline with intra-epithelial carcinoma) [9]. Most of the borderline BTs are detected in the first stage and have a favorable progression after the surgical treatment, with a survival rate of five years in about 99% of cases, and of 10 years in about 97% of cases [10].

Conclusion

We describe a case of borderline BT associated with a concomitant mucinous ovarian tumor. The positive diagnosis was done after surgery through the classical histopathological evaluation associated with immunohistochemical examinations. The progression after surgery was a favorable one, there were not detected any abdominal relapses during the ultrasound examination.

Informed consent

Written informed consent was obtained from the patient for the publication of this case report.

Conflict of interest

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Magnetic resonance imaging findings of a patient with bilateral submandibular gland aplasia

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ABSTRACT

Aplasia of the major salivary glands are very rare, and commonly occurs at the parotid gland. Patients are usually asymptomatic and diagnosed incidentally. But dry mouth, difficulty in swallowing, dental problems may be seen. Also mass complaints may occur due to compensatory hypertrophy of the other major salivary glands. Herein, we presented the magnetic resonance imaging findings of a very rare case of bilateral submandibular gland aplasia with compensatory hypertrophy of sublingual glands.

Eur Res J 2018;4(2):126-128

Keywords: salivary glands, magnetic resonance imaging, compensatory hypertrophy, submandibular gland aplasia

Introduction

Aplasia of the major salivary glands is a very rare situation of which the incidence is not accurately known. Approximately 40 cases with congenital absence of the submandibular gland have been reported in the literature [1]. Although the etiology is not proven, it has been thought to occur due to a defect in fetal development [2]. In addition; submandibular gland aplasia can be seen in Lacrimo-aurikulo-dento-digital syndrome (LADD), Mandibulofacial Dysosteosis (Treacher- Collins Syndrome), Ectodermal Dysplasia with lacrimal-thyroid aplasia or dysplasia [3, 4].

In this article, we presented magnetic resonance imaging (MRI) findings of bilateral submandibular gland aplasia with compensatory hypertrophy of sublingual glands in a 21-year-old woman.

Case Presentation

A 21-year-old woman referred to ear-nose and throat clinic with long-term xerostomia and teeth complaints. In physical examination, a solid-mobile mass in submandibular region was found by palpation. There was no significant data in laboratory, so an

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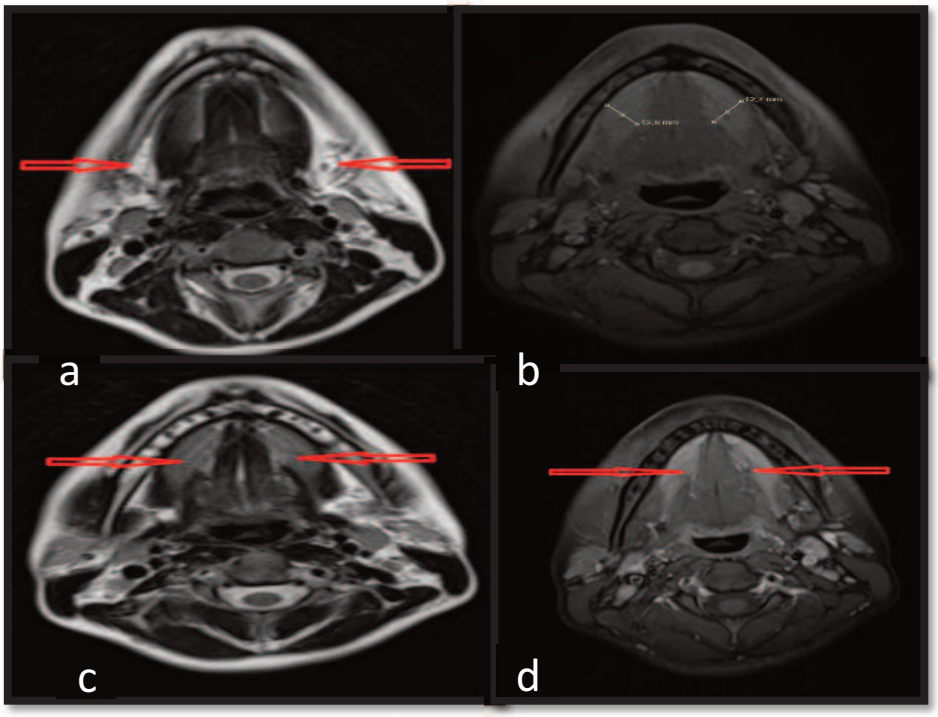

Figure 1. (a) Both bilateral submandibular glands were not seen in their normal localization- T2A view (arrows), (b) Sublingual gland thickness 12.8 mm in right and 12.7 mm in left- T1A view, (c) Sublingual glands T2A view and (d) Sublingual glands T2A contrasted fat saturation view (bidirectional arrows).

ultrasound screening was planned. Absence of submandibular glands and hypertrophy of sublingual glands were detected by ultrasound.

After that a contrasted neck MRI were applied and no submandibular gland were seen in normal region either (Figure 1a). Besides we measured the thickness of sublingual glands 12.8 mm in right, and 12.7 mm in left (Figures 1b, 1c and 1d). Whereas Sumi et al. [5] reported normal thickness of those as 5mm or less. Finally, the patient was diagnosed as bilateral submandibular gland aplasia with compensatory hypertrophy of sublingual glands.

Discussion

Aplasia of salivary glands is observed rarely, and the reported number is about 40 cases [1]. Aplasia of submandibular glands may be unilateral or bilateral, total or partial [6]. The etiology is claimed to be a defect of major salivary gland and oral ectoderm proliferation and migration between 4-8 weeks during fetal development. It can be associated with 1st and 2nd branchial arch anomalies. Submandibular glands maturate after parotid gland. The maturation of sublingual and minor salivary glands is later [7].

Half of the cases are asymptomatic and may be detected incidentally. Xerostomia, dysphagia and tooth problems are major complaints in symptomatic patients. In some patients, other salivary glands may have a compensatory hypertrophy as the case presented in this report, and they can be evaluated as mass [1].

Anamnesis and bimanual palpation are very important. Ultrasound, computed tomography, MRI, sialography, even scintigraphy can be applied for diagnosis [8]. By these imaging methods, absence of submandibular glands has to be proven and associated sublingual gland hypertrophy may be observed; the tumors originating from the floor of mouth should to be excluded. Radiologists must be familiar to this benign situation to prevent unnecessary biopsies. Clinicians must be very careful about branchial arc anomalies associated to major salivary gland aplasia. Sjogren syndrome should be considered in the differential diagnosis, and it can be excluded if the submandibular glands are detected in normal localization. The tumors of mouth floor also must be take into account. They are mostly diagnosed by imaging; however, biopsy and pathological evaluation should be performed in some cases [9].

Conclusion

Finally, major salivary gland aplasia and especially bilateral submandibular gland aplasia should be kept in mind in patients with the symptoms

such as neckmass, teeth problems, dysphagia and mouth dryness.

Informed consent

Written informed consent was obtained from the patient for the publication of this case report.

Conflict of interest

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Left atrial myxoma combined with coronary artery disease in an elderly patient

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ABSTRACT

Cardiac myxoma associated with coronary artery disease is a rare pathology in an elderly patient. Herein we present an 81-year-old woman undergoing simultaneous surgical treatment for left atrial myxoma combined with coronary artery disease. The postoperative course of the patient was uneventful. The initial removal of mass should be performed to prevent systemic embolization of tumor fragments.

Eur Res J 2018;4(2):129-131

Keywords: Left atrial mass, coronary artery disease, elderly

Introduction

Atrial myxomas are accounting for nearly half of primary cardiac tumors in adults [1]. Approximately 75-90% of cardiac myxomas are located in the left atrium, mainly adhered to the atrial septum near the fossa ovalis [2, 3]. In most cases they encountered in female patients with 40-60 years old [1]. The size of the myxoma is directly proportional to the severity of the patient's symptoms [4]. Coexistence of coronary artery disease and myxoma is a rare condition and, it is reported in a limited number of case reports [5-8]. We describe a rare case with simultaneous left atrial myxoma and coronary artery disease in an elderly woman with shortness of breath and chest pain.

Case Presentation

An 81-year-old woman admitted to hospital with shortness of breath and chest pain. Upon physical examination, the patient had a heart rate of 78 beats/min, and a regular blood pressure of 170/70 mmHg. In the clinical history, she had undergone coronary angiography 3 years ago and coronary artery disease had been detected. There was no previous echocardiography report.

The systemic examination of the patient was normal. The electrocardiogram was also normal. Echocardiographic evaluation identified left ventricular apical akinesis with an ejection fraction of 45% and showed a left atrial mass 38×32 mm in sizes attached to the interatrial septum of the left atrium without obstruction.

Coronary angiography showed plaques with non-critical stenosis in the left circumflex coronary artery, an ostial stenosis of 90% of the right coronary artery, but 90% stenosis at the proximal portion of the left anterior descending coronary artery (Figure 1).

After general anesthesia surgery was performed via a median sternotomy. The aorta, and superior and inferior venae cava were cannulated, and

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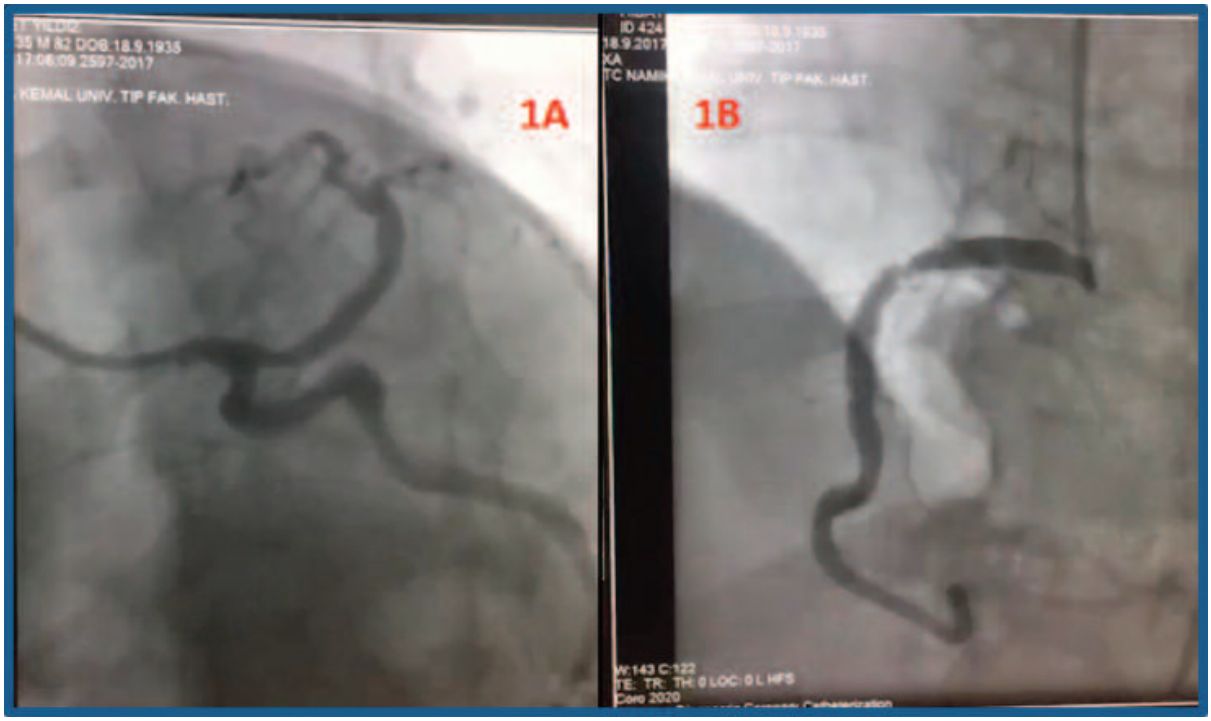

Figure 1. Coronary angiography shows demonstrating severe stenosis in the left anterior descending coronary artery (A) and the right coronary artery (B).

cardiopulmonary bypass with moderate hypothermia (28-30°C) was instituted. To minimize the risk of developing perioperative embolism the heart was gently handled during the operation. First the left atrial mass excised via interatrial groove approach (Figure 2). Excision of the tumor was performed with its pedicle originated from the left side of the atria. We performed coronary artery bypass grafting after the closure of the left atriotomy. For coronary revascularization of left descending coronary artery the internal thoracic artery was used and a saphenous vein graft anastomosed to aorto-right coronary position. The postoperative course was uneventful without any complication.

Discussion

We have presented a rare case of concomitant atherosclerotic coronary artery disease and left atrial myxoma. Moreover, this patient was the oldest patient

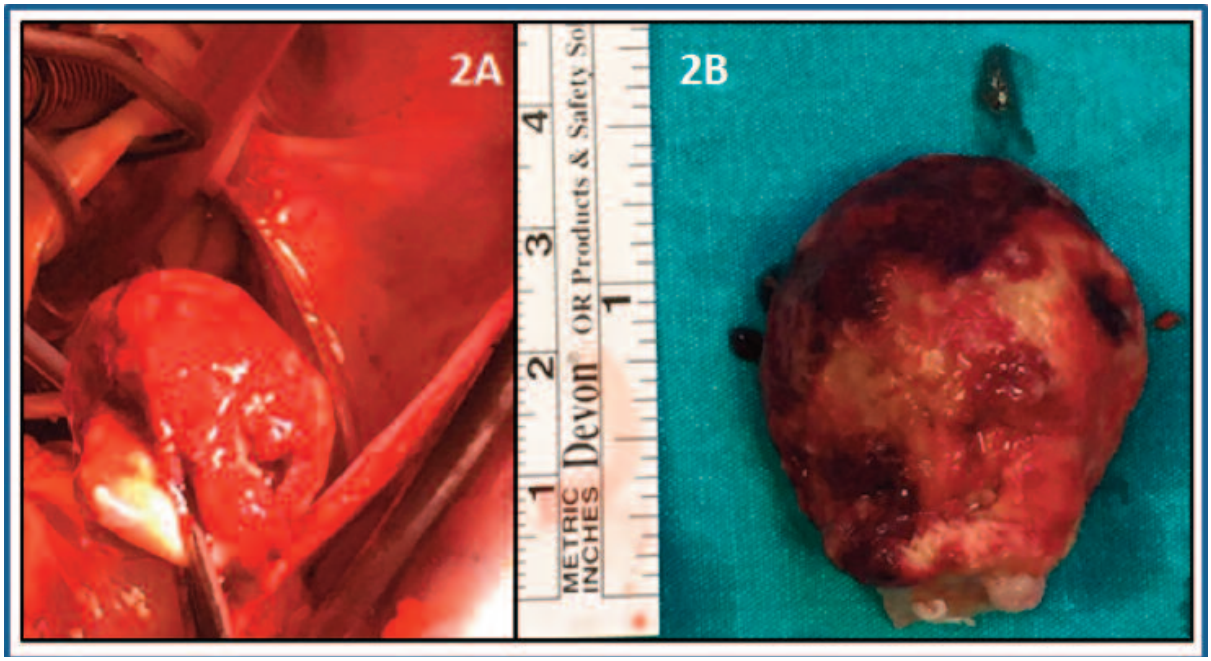

Figure 2. Appearance of the atrial mass during surgical excision (A). Macroscopic view of the surgically removed myxoma (B).).

with coronary artery disease associated myxoma according to literature review. Atrial myxomas are the most common primary heart tumors [2, 3]. However, the incidence of atrial myxomas ranges between 0.5 and 1 per million of population/year and mean age of presentation has been reported between 40 to 60 years [1].

Left atrial myxoma may or may not produce characteristic findings on physical examination. The myxoma symptoms depend on mitral valve obstruction or distal organ embolization [4]. Embolization is generally seen in central nervous system. But other organs such as the liver, spleen, kidney, retina, coronary vessels, and distal arterial tree can be affected [4]. Chest pain and dyspnea were the major symptoms of the patient. Clinical findings were thought to be the result of partial cardiac obstruction in addition to coronary artery disease. In case of acute coronary syndromes and left heart myxoma, coronary embolization should always be considered [5]. The incidence of coronary embolization of myxomas is 0.06%. There was no evidence of major distal embolism in our patient. She had angina and shortness of breath at the same time. So we decided to operation because of clinical deterioration.

The prevalence of coronary atherosclerotic disease in patients with myxoma ranges between 20.3% and 36.6% [6-9]. In this report the patient had coronary artery disease according to coronary angiography 3 years ago. But myxoma not reported in previous echocardiography note.

Conclusions

Coronary angiography is required in elderly patients who will undergo other cardiac operations because of the risk of synchronous coronary artery disease. Similarly, echocardiography is also required in patients with coronary artery disease, because of the risk of synchronous cardiac lesion, as seen in our case.

Informed consent

Written informed consent was obtained from the patient for the publication of this case report.

Conflict of interest

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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An arteriovenous malformation as a rare cause of sciatic neuropathy: a case report

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ABSTRACT

Sciatica is characterized by pain and discomfort occurring frequently with impingement at the level of spinal nerve along the regions innervated by the sciatic nerve. Compression and irritation of the nerve often occurs with a spinal cause such as lumbar disc hernia or spinal stenosis. Compression of the nerve by an arteriovenous malformation is very rare among the causes leading to non-discogenic sciatic neuropathy. Herein, we reported our case with clinically and electrophysiologically typical sciatic neuropathy and treated by us surgically. To our knowledge, cases with arteriovenous malformation -caused sciatica were limited in the literature review. Electromyography should be performed to exclude the nerve compression due to rare causes such as vascular causes which may lead to sciatic neuropathy in patients with sciatic distribution symptoms and signs, after initial negative spine imaging.

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Keywords: Sciatic neuropathy, vascular, electromyography, arteriovenous malformation

Introduction

Sciatica is defined as pain or discomfort along the regions innervated by the sciatic nerve [1, 2]. Although sciatica is most often radicular in origin, new radiating pain despite negative lumbar imaging studies warrants consideration of such nondiscogenic origins as plexopathy or neuropathy. Infrequent causes include benign or malignant tumors, infections, mechanical entrapments, and vascular causes [3]. Arteriovenous malformations (AVMs) among vascular causes are frequently seen in head and neck region and it is too rare to cause sciatic nerve compression by them. AVMs are the high-flow lesions which are aggressively growing and causing tissue destruction [4]. They are usually asymptomatic and seen in the brain, lungs and lower extremities. They rarely cause peripheral neuropathy.

Herein, we reported our case with sciatic neuropathy due to compression of AVM. Lumbar spinal magnetic resonance imaging (MRI) of the patient was negative, but clinical and

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electromyography (EMG) findings were supporting sciatic neuropathy. Surgical exploration was performed and then total excision was performed after ligation of the feeders and drainage veins of AVM compressing the sciatic nerve. External and limited internal neurolysis was performed. Pain of the patient was improved postoperatively and he had no pain at first year follow-up.

Case Presentation

A 53-year-old man presented with persistent left sided, radiating leg pain. The patient defined the pain present for 6 months in the left buttock, posterior and lateral thigh and lateral calf. He had no history of a known disease but he defined an intramuscular injection performed in left gluteal region 8 months ago. Laseque and straight leg raise test was negative. Weakness was determined in foot dorsiflexion and plantar flexion (3/5). Numbness in a sciatic distribution from the lower buttock toward the foot. An ankle jerk was decrease. Mild foraminal protrusion was determined at the L4-5 level on MRI (Figure 1a and 1b). Since the clinic could not be explained by MRI findings, EMG was performed. Nerve conduction studies showed reduced peroneal (recording over the extensor digitorum brevis and tibialis anterior) and tibial compound muscle action potential (CMAP). Sensory studies demostrated abnormality sural and superficial peroneal sensory nerve action potential (SNAP). Electromyography showed neurogenic abnormalities in the tibialis anterior, medial gastrocnemius and the short head of the biceps femoris. MRI was not performed because of strong suggestion of sciatic neuropathy by clinical presentation and electrophysiological studies. Given these findings, we considered sciatic neuropathy which could be as a late complication of gluteal intramuscular injection. We decided to perform a left sciatic exploration. Under endotracheal general anesthesia, the patient was placed in the genupectoral position. First, the posterior inferior iliac spine and the site 1 inch behind the greater trochanter tip were identified. Second, a longitudinal curved skin incision was made between these two points distally and extended about 4 cm along the femoral shaft. After muscle dissection, AVM was seen and it was located on the sciatic nerve and compressing it. The AVM was originated from the superior gluteal artery and drained to inferior gluteal vein. Engorged, purple veins and one prominent draining vein as well as small arterial feeders were noted. After ligation of the feeders and draining veins one by one, AVM was excised totally (Figure 2a and 2b). Surgical exploration with external neurolysis as well as limited internal neurolysis of the sciatic nerve. After surgery, the patient's symptoms resolved, and she could sit for about 1 hour and walk without support 3 months later, the visual analog pain scale score had dropped from 10 to 2. At the final

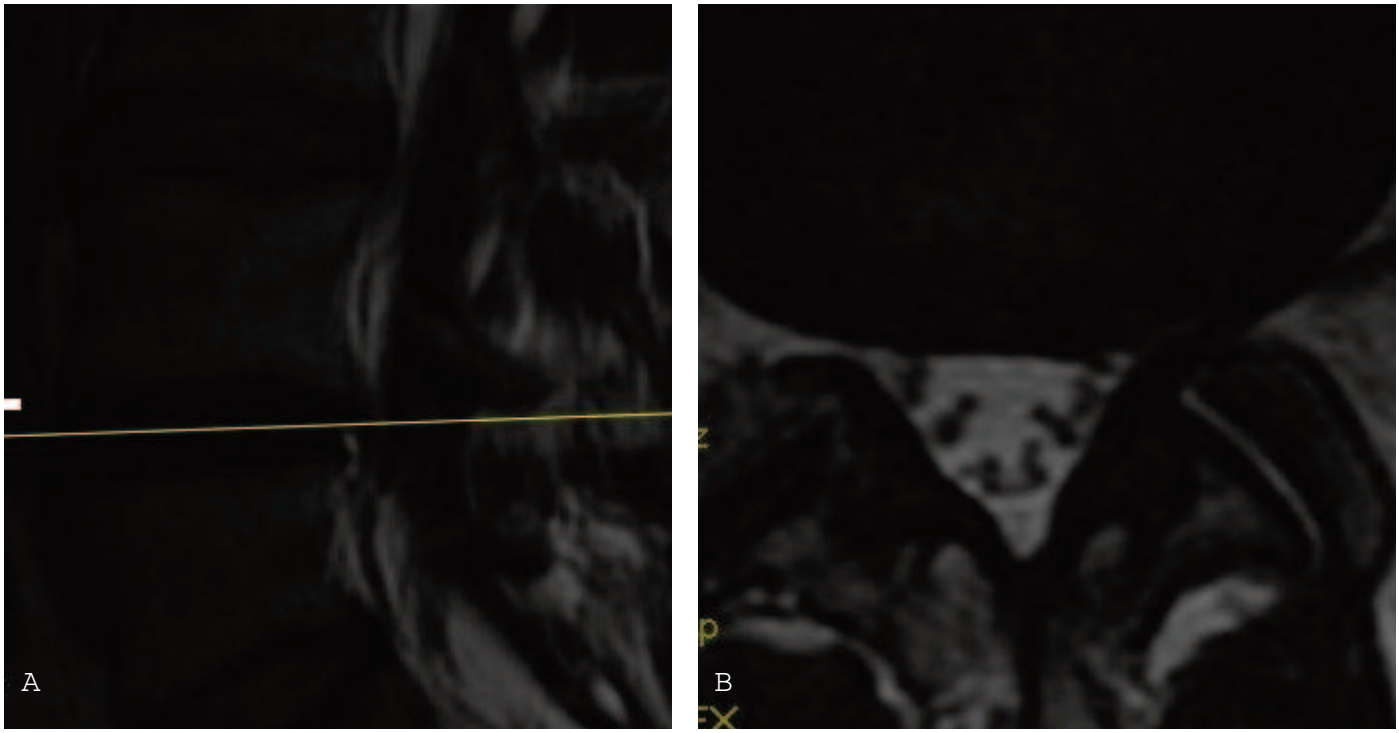

Figure 1. Mild foraminal protrusion was determined at the L4-5 level on MRI (a and b).

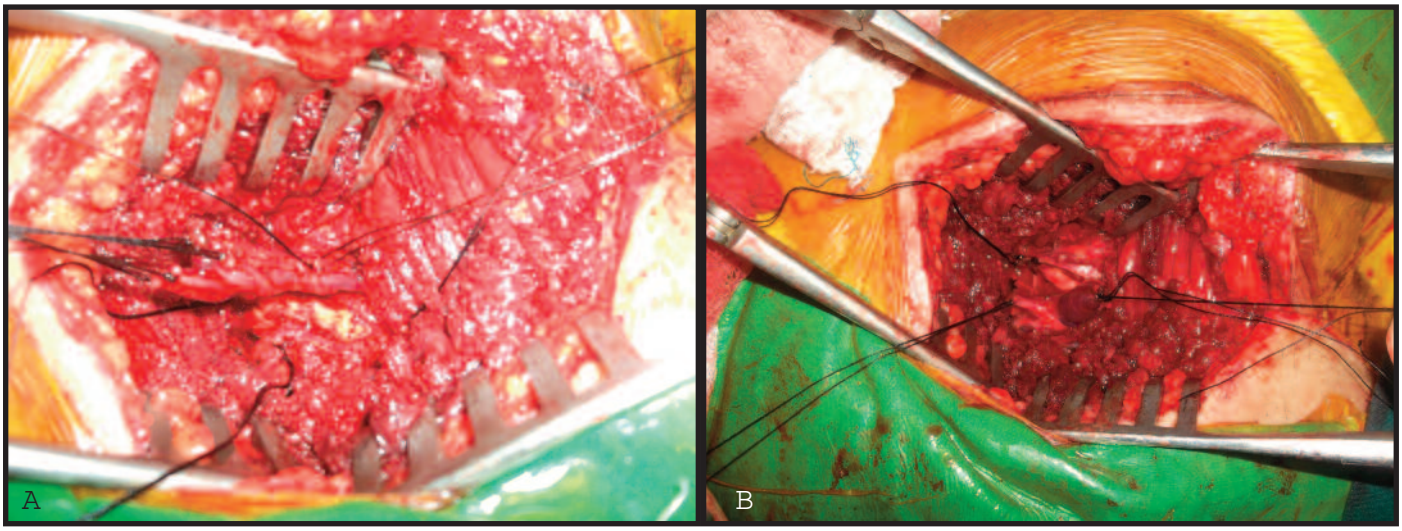

Figure 2. Engorged, purple veins and one prominent draining vein as well as small arterial feeders were noted (a). After ligation of the feeders and draining veins one by one, AVM was excised totally (b).

follow-up evaluation 12 months later, the patient reported no recurrence of his pain.

Discussion

The sciatic nerve has 2 divisions: the superficial and lateral peroneal nerves, and the medial tibial nerve. The sciatic nerve trunk innervates hamstrings and the distal adductor magnus. The peroneal and tibial nerves supply the anterior and posterior leg and intrinsic foot musculature. Through sensory branches of the tibial (sural, medial, and lateral plantar; and calcaneus) and the superficial peroneal nerves, it supplies sensation to the foot and posterior lower leg. As a consequence, sciatic neuropathies present with weakness below the knee and numbness over the calf and foot, and the ankle reflex is usually absent [1, 5]. Although the vast majority of sciatica is due to degenerative causes and is radicular in nature, various other causes are to be considered when spine imaging is negative [3]. Sciatic neuropathy is distinctly uncommon and is associated with a limited differential diagnosis [6]. Hip or femoral fractures and the complications of the surgeries of these fractures [6], tumors as another cause of common sciatic neuropathy (neurofibroma, schwannoma, neurofibrosarcoma, lipoma and lymphoma), infections and large Baker's cyst in the popliteal may compress the sciatic nerve [1, 7]. Sciatica has been reported, although rarely, to be related to vascular lesions along the course of the sciatic nerve from the pelvis to its bifurcation [6, 8-10]. Other vascular lesions discussed as causes of sciatica are anatomic anomalies such as a sciatic artery or vein, gluteal varicoses, venous thrombosis, hemangiomatosis in Klippel-Trenaunay syndrome, and venous or capillary hemangioma [2, 3, 6, 7, 11]. Inferior gluteal artery aneurysm, usually caused by trauma, is more common in neurovascular compression mimicking lumbosciatic pain [12]. Vascular causes of peripheral neuropathy are extremely uncommon and require a focused workup. AVMs are uncommon vascular lesions formed by multiple abnormal communications between the arterial and venous systems without an intervening normal capillary network [4]. The etiology of these lesions has been a subject of controversy, although it is generally agreed that the greater part of these lesions are congenital, with a few that are acquired after trauma or surgery [4]. AVMs are high-flow lesions, characterized by aggressive growth and tissue destruction. The majority of AVMs are asymptomatic and affect the brain, lung, and the lower extremities. Lower extremities AVMs most commonly manifest with dermatological signs including discoloration and swelling. Development of peripheral neuropathy due to AVM compression is very rare [3, 5, 11].

Symptoms of the case presented by us were supporting the spinal etiology, but no significant pathology was determined in spinal imaging of the patient. Findings supporting the sciatic neuropathy were seen in the EMG performed (decreasing the CMAP and SNAP potentials in the nerve conduction study and neurogenic abnormalities in the tibialis anterior, medial gastrocnemius and the short head of the biceps femoris). We considered that the neuropathy of the patient with history of gluteal injection performed 8 months ago could be late complication of

injection injury firstly [13]. We explored left sciatic nerve. After the ligation of AVM's feeders and drainage veins compressing the nerve, we performed total excision, external neurolysis and limited internal neurolysis. Pain of the patient improved completely after the surgery.

Endovascular embolization of the AVMs causing sciatic neuropathy is a method of treatment that can be performed, but surgical excision is still successful since these cases are very rare and loss to long-term follow-up [4, 9]. Development of sciatic neuropathy due to a vascular etiology is a very rare condition. When the literature is scrutinized, the number of the cases with sciatic neuropathy developed due to compression of an AVM does not exceed [2].

In the cases presented with sciatic clinic but having negative spinal imaging, peripheral nerve MRI and/or EMG should be necessarily performed to exclude the other reasons caused sciatic neuropathy. MRI was not performed because of strong suggestion of sciatic nerve compression at the proximal region by clinic and electrophysiological studies.

Conclusion

With this rare case, we aimed to emphasize the necessity of presence of a pathology compressing the nerve and EMG and radiological investigation of the nerve in the cases with sciatic neuropathy clinic but having negative spinal imaging. We discussed how AVMs could be encountered in the clinic as a rare reason caused sciatic nerve compression and their treatments.

Informed consent

Written informed consent was obtained from the patient for the publication of this case report.

Conflict of interest

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Metachronous infiltration of bone marrow due to breast cancer and multiple myeloma

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ABSTRACT

Multiple primary tumors are rarely seen in clinical practice. Cases of metastatic breast cancer associated with multiple myeloma were rare in the literature. It is believed that simultaneously observed bone marrow involvement due to breast cancer and multiple myeloma is sporadic. We present a 49-year-old female patient with metastatic breast cancer who developed multiple myeloma during breast cancer treatment.

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Keywords: Bone marrow involvement, breast cancer, multiple myeloma, metachronous malignancy

Introduction

Multiple myeloma is a malignant disease of plasma cells. It is a relatively rare disease. It forms 1% of all cancer cases and 10% of all hematological malignancies [1]. Other solid organ tumors rarely accompany multiple myeloma. Breast cancer is the most frequently seen cancer in women worldwide. Its relation with colon, vulva, lung, larynx, liver, uterus cancers has been identified in the literature [2]. A case with a history of breast cancer came to internal medicine clinic offering findings remarkable for multiple myeloma.

Case Presentation

A 49-year-old female patient with no significant history except hypertension came to her physician with a complaint of left breast mass and the patient underwent a tru-cut needle biopsy one year ago. A diagnosis of invasive ductal carcinoma exhibiting both estrogen- progesterone receptor positivity was established. PET CT demonstrated thoracic vertebral involvement. Clinically, the patient appeared to have a stage 4 breast carcinoma. She began a chemotherapeutic regimen of cyclophosphamide (600 mg/m^2), epirubicin (60 mg/m^2) and 5-fluorouracil (500 mg/m^2). She received nine cycles of this regimen followed by the left modified radical mastectomy and lymph node dissection.

After surgery, the patient received two more cycles of chemotherapy. Since the patient stated that she had been fatigued and having palpitation, she came to internal medicine clinic in our hospital with concerns regarding these symptoms. The patient was electively admitted to our clinic for evaluation. Vital signs were blood pressure 130/85 mm/Hg, temperature 36.7°C, respiratory rate: 18/min, and heart rate: 78 beat/min.

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Cardiovascular and pulmonary examinations were within normal limits. ECG and chest x-ray were normal. Routine laboratory analyses were follows: hemoglobin 7.6 mg/dL, white blood cells 5,400/µL, platelet counts 234,000/µL, erythrocyte sedimentation rate 84 mm/h, sodium 129 mm/L, potassium 4.68 mm/L, calcium 9.5 mm/L, albumin 3.1 g/L, globulin 7.6 g/L, serum LDH 299 IU/L, serum ALP: 789 IU/L, and uric acid 6.8 mg/dL. Liver, kidney, and thyroid function tests were normal. Urine analysis revealed pH: 6, density 1,025, protein 1+, leukocyte 2+, bilirubin, and glucose (-). Peripheral smear showed hypochromia, anisocytosis, and minimal poikilocytosis without atypical cells.

Due to the reduction of albumin/globulin ratio and symptomatic anemia, serum immunoglobulin profile was requested. Serum immunoglobulin profile revealed IgG: 6,190 mg/dL, IgM: 10.2 mg/dL, IgA: 9.59 mg/dL, and IgE: 4mg/dL. Serum immunofixation test revealed monoclonal gammopathy (serum kappa light chain: 1,457 mg/dL, serum lambda light chain: 183 mg/dL). Then the patient's anamnesis was deepened, it was determined that there was a recurring back pain for the last one month in spite of analgesic treatment. Tumor invasion of the thoracic vertebra was detected in PET CT after the patient was diagnosed with breast cancer, but the lumbar vertebra was the usual appearance.

New lytic lesions that were not observed before chemotherapy was detected in a linear graph of lumbar vertebrae taken to illuminate the etiology of the patient's back pain. (L2-L4). On the initial assessment of the patient appeared to have hyponatremia, anemia, hypergammaglobulinemia, monoclonal gammopathy and lytic lesions on radiography. A full chemistry panel was significant for a prominent increase in the serum globulin and an elevated IgG. These aspects of her hyponatremia were highly suggestive for pseudohyponatremia to paraproteinemia.

Histopathologic examination of the bone marrow biopsy revealed CD138+ plasma cells with atypical morphology and tumor tissue with epithelial cells with large hyperchromatic nuclei (Figures 1-3). A presumptive diagnosis of simultaneously observed bone marrow involvement due to breast cancer and multiple myeloma was made. Hematology was consulted. A review of the pathology revealed multiple myeloma and the patient was referred to hematology clinic. The decision to treat this patient was based upon bone marrow involvement by atypical plasma cells, monoclonal gammopathy. The consultants on hematology recommended chemotherapy for multiple myeloma. The patient underwent nine cycles of bortezomib-dexamethasone treatment. Full remission was achieved.

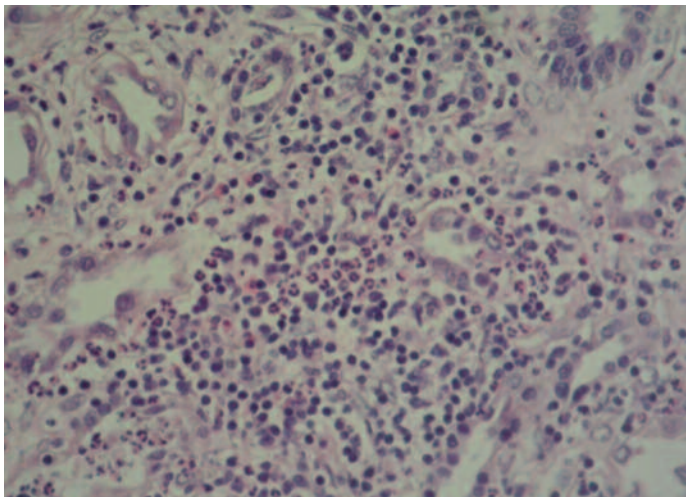

Figure 1. Areas of carcinoma in bone marrow by immunohistochemical test

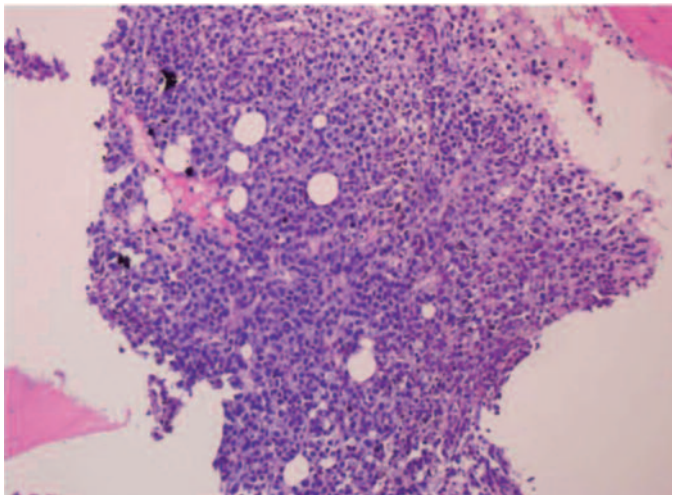

Figure 2. Plasma cells of multiple myeloma in bone marrow. H&E × 200

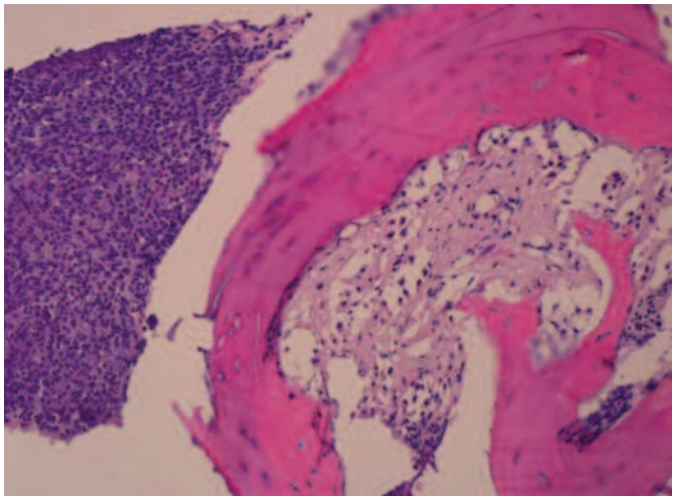

Figure 3. Plasma cells and areas of carcinoma in bone marrow. H&E × 200

Discussion

It is a rare situation to see multiple carcinomas in the same case. It is also very rare to see bone marrow involvement due to breast cancer and multiple myeloma in the same case.

Multiple primary carcinoma descriptions are provided with the following criteria: each malignant tumor must bear histological features; they must be different from each other histologically; exclusion must be done as they are not metastasis of each other [3].

The synchronous tumor is a situation when the diagnosis of a second tumor is established at the same time or in the following six months. If this rule is not provided, the second tumor is called as metachronous [4]. In our case also offered these three criteria, in addition to this, we evaluated as metachronous tumors in our study because multiple myeloma occurred one year after the diagnosis of breast cancer. Family history, genetic predisposition, previous chemotherapy and radiotherapy history are essential factors for various tumor developments [5]. We did not detect family history, radiotherapy history or a genetic mutation. She had received a chemotherapeutic regimen of cyclophosphamide, epirubicin, and 5-fluorouracil. She received 11 cycles of this regimen. Plasma cell dyscrasia after chemotherapeutic regimen with alkylating chemotherapeutic agents especially like cyclophosphamide has been reported in the literature [6].

Multiple primary carcinoma cases were detected in 2.4% of 19,252 patients diagnosed with breast cancer in a study conducted in Italy [7]. Kılçıksız *et al.* [8] identified 297 primary tumor cases (1.47%) among 20,290 cancer patients. This ratio was 1.74% in breast cancer cases (38/2,186). It was reported that breast cancer could be associated with colon, vulva, lung, larynx, liver, and cancer of the cervix [2]. Although differences in the cases of breast cancer, multiple primary tumors are often seen in the elderly. Various primary tumors accompanying breast cancer are mostly seen in young ages and this rate decreases over 65 years of age [9]. Our patient was 49 years old, is compatible with this situation. Some previous case reports are showing the simultaneous existence of a solid tumor and multiple myeloma. The simultaneous presence of a solid tumor and multiple myeloma was reported as 3% by Stegeman *et al.* [10]. Sakai *et al.* [11] reported an association between multiple myeloma with clear cell renal carcinoma and emphasized the possible role of growth factors like IL-6. Kherfani *et al.* [12] reported a synchronous hormone receptor-positive breast cancer and multiple myeloma case with the involvement of thoracic and lumbar spinal cord. Sehgal *et al.* [13] reported a case of prostate adenocarcinoma and multiple myeloma with synchronous infiltration of bone marrow. Increased release of growth factors like IL-6, IGF-1 derived from multiple myeloma has been reported to trigger other malignancies. Demir *et al.* [14] reported a case of synchronous stomach adenocarcinoma and multiple myeloma.

Conclusions

The simultaneous existence of breast cancer and multiple myeloma are rarely reported. The presence of some laboratory signs and symptoms in patients with metastatic malignancy can be stimulating regarding synchronous or metachronous malignancies. Further studies are needed to determine the underlying etiologies and genetic factors in synchronous or metachronous malignancies.

Informed consent

Written informed consent was obtained from the patient's family for the publication of this case report.

Conflict of interest

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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