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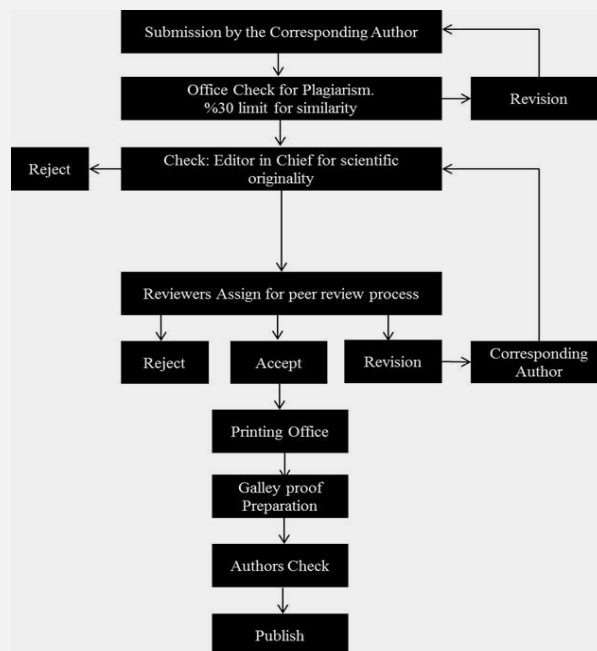
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Contents

Could sP-Selectin and sICAM-1 be potential biomarkers in status epilepticus?

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Abstract

Objective: To investigate whether soluble P-selectin (sP-selectin) and soluble intercellular adhesion molecule-1 (sICAM-1) might be potential biomarkers that predict the course and prognosis of status epilepticus (SE).

Patients and Methods: Fourty-two adult patients with SE between February 2012 and December 2013 were included in the study. Clinical and demographic features of the patients were recorded and surviving patients were followed for 13.6 ± 4.6 months. Serum sICAM-1 and sP-selectin levels were measured during SE or within 24 hours of SE, and compared with 28 subjects in the control group.

Results: Levels of serum sP-selectin and sICAM-1 were higher in the SE group compared with the control group (P: 0.04 and P: 0.02, respectively). It was shown that higher levels of serum sICAM-1 correlated with poor outcomes (P: 0.017) and "ROC curve" analysis showed that levels higher than 457 ng/mL predicted poor outcomes with 71% sensitivity and 68% specificity. Levels of serum sP-selectin did not correlate with outcomes. Subgroup analyses revealed levels of serum sICAM-1 were significantly higher in the epilepsia partialis continua (EPC) group compared with the control group (P: 0.012) and levels of serum sP-selectin were not different between subgroups and controls. Levels of serum sP-selectin and sICAM-1 didn't differ between subgroups of SE and different etiologies.

Conclusion: Higher levels of serum sICAM-1 may predict poor outcome in SE, as a result sICAM-1 may be used as a biomarker of the prognosis of SE in clinical practice. The production of sICAM-1 may increase particularly in EPC. However, no correlation was found between etiology of SE and serum level of sICAM-1, even in patients with EPC. Serum level of sP-Selectin is not an appropriate biomarker for the prognosis of SE. Serum levels of sICAM-1 and sP-selectin are not appropriate biomarkers of refractory SE.

Keywords: status epilepticus, biomarker, sP-selectin, sICAM-1

Introduction

Biomarkers of epileptogenesis and ictogenesis may exist and in turn, these might be used to predict the onset of epileptic episodes and determine the presence and possible extent of tissue that may predispose to spontaneous episodes. These biomarkers also have the potential to be used to evaluate prognosis once the clinical episode has settled and to create animal models for more cost-effective screening of potential antiepileptogenic and antiseizure drugs and devices, and reduce costs in potential antiepileptogenic and antiseizure clinical studies by enriching the study population with patients at high risk for epilepsy. Biomarkers of epilepsy might be found via imaging modalities, electrophysiologic measurements, and molecules measured in tissue, blood, or cerebrospinal fluid (CSF) (1).

Brain injury that results from status epilepticus (SE) is mainly due to the excitotoxic damage caused directly by seizures (2). Biomarkers of brain injury would be useful in determining critical seizure duration in terms of detecting neuronal injury and defining epilepsy subtypes that may require more aggressive treatment, thus serving as prognostic markers. Several biomarkers have been correlated to epilepsy and brain injury. Brain-derived neurotrophic factor (BDNF), (3) myelin basic protein (MBP), (4) and glial fibrillary acidic protein (GFAP), (4, 5) neurofilament heavy chain protein and heat shock protein (HSP-70) (6) as well as various enzymes (enolase, aldolase, pyruvate kinase, lactate dehydrogenase, creatine phosphokinase), (7) metalloproteases, (8) tau protein, (9) neuron-specific enolase (NSE) (10) and S100B protein (11) in particular, have been investigated.

It has recently been demonstrated that modification of astroglial function, immune/inflammatory reactions, and impairment of blood brain barrier also contribute to brain injury resulting from seizures (12-14).

Cellular adhesion molecules are known to play important roles in inflammatory pathologies (15-17) particularly including the pathogenesis of atherosclerosis (18) and several neurologic diseases such as ischemic stroke, multiple sclerosis, and Alzheimer's disease (19) by mediating the migration of immune cells into the inflamed tissue. Adhesion molecules on endothelial cells and immune cells are termed soluble cellular adhesion molecules when shed into the circulation, and might be determined using an enzyme-linked immunosorbent assay (ELISA) (20).

Animal studies have demonstrated that seizures cause an inflammatory wave, which results in the increased synthesis of adhesion molecules, (12, 21, 22) and increased soluble intercellular adhesion molecule-1 (sICAM-1) synthesis via intraventricular injection of kainic acid (23). Furthermore, administration of monoclonal antibodies aimed at adhesion molecules following pilocarpine-induced seizures has provided a marked reduction in seizures (22). However, few human studies have investigated the relationship between seizures and adhesion molecules (24-27).

The objective of this study was to compare levels of sP-selectin and sICAM-1 in patients with SE and healthy controls to determine whether these could be potential biomarkers that predict the course and prognosis of SE.

Material and Methods

1 Patients and Clinical Data

Fourty-two adult patients who were diagnosed as having SE following referral to the Emergency unit of the Neurology Department or during their hospitalization in other clinics of Istanbul Medical Faculty of Istanbul University between February 2012 and December 2013 were included in the study, and their demographic and clinical features in addition to early and late prognosis were recorded prospectively. At first there were 50 patients, but 8 patients who had cerebrovascular disease were excluded because serum levels of adhesion molecules are known to be affected by atherosclerotic risk factors (28). More patients would be expected to be included in such a study but written informed consents could only be obtained for 50 patients from them or their close relatives when the patients were in SE. The study was approved by the Ethics Committee of Istanbul Medical Faculty of Istanbul University prior to the performance of any study procedures. All patients and volunteering controls were informed about the study outline and examinations, and written informed consent was obtained from all participants or their relatives if they were unconsciousness due to SE.

2 Blood Samplings

Blood samples were drawn from all subjects in the study and control groups during or within 24 hours of SE, and centrifuged for 10 minutes at 3000 revolutions per minute to obtain serum. Serum samples were then stored at -80°C until required for analysis. Levels of sP-selectin and sICAM-1 were measured in the serum samples using the commercial ELISA kits (Bender MedSystems, Vienna, Austria) at the Central Biochemistry Laboratory of the Department of Biochemistry of Istanbul Medical Faculty of Istanbul University.

Levels of sP-selectin were measured in 42 patients with SE and 26 controls, and sICAM-1 levels were measured in 40 patients with SE and 28 controls. The discrepancy between the number of patients and control samples was due to a lack of serum samples. The control group consisted of age- and sex-matched healthy volunteers who worked in our hospital or relatives of patients who were hospitalized. Control population did not have any inflammatory or infectious disease, neoplasms, hypertension, diabetes mellitus, chronic hepatic and renal disfunction, cerebrovascular disease which may alter serum levels of soluble adhesion molecules.

3 Seizure Types

Generalized convulsive status epilepticus (GCSE) was defined as one seizure or two seizures, without attainment of consciousness in-between, lasting longer than 5 minutes (29). The diagnosis of epilepsia partialis continua (EPC) was established by the observation of a seizure with contractions involving only focal parts of the body and lasting at least 30 minutes; consciousness is often preserved although various degrees of altered consciousness might be observed (30). The diagnosis of non-convulsive SE (NCSE) was established through an electroencephalogram (EEG) (31). Refractory SE (RSE) was defined as SE refractory to treatment with first-line benzodiazepines (BZD) and any of the second-line antiepileptic drugs (AED) including phenytoin (PHT), valproic acid (VPA), levetiracetam (LEV), and phenobarbital (PB) (32).

4 Treatment

Patients with GCSE were initially treated with intravenous (i.v.) 10 mg diazepam (Diazem®; 10 mg given over 2-5 min). If seizures continued, another 10 mg diazepam was administered and followed by either one or two of i.v. LEV (Keppra®; 20-60 mg/kg given over 10 min), PHT (Epitoin®, Epanutin®; 15-20 mg/kg given at a rate of 50 mg/min), or VPA (Depakin®; 20-40 mg/kg given over 10 min). Refractory GCSE was treated by anaesthetic doses of barbiturates (Pentothal Sodium® (thiopental); 2-3 mg/kg bolus followed by 3-5 mg/kg/h infusion), midazolam (Dormicum®; 0.2 mg/kg bolus followed by 0.1-0.4 mg/kg/h infusion) or propofol (Propofol®, Pofol®; 3-5 mg/kg bolus followed by 5-10 mg/kg/h infusion); the anaesthetics were titrated against an electroencephalogram burst suppression pattern for at least 24 h. Patients with NCSE and EPC were initially treated in same manner as patients with GCSE. But if they became refractory, further non-anaesthetising i.v. substances such LEV, PHT or VPA were administered instead of anaesthetics. In patients with medical co-morbidities and multiple drugs usage, we preferred LEV instead of PHT and VPA. Brand names of the drugs used in Turkey were given with their doses in brackets.

5 Prognosis

Prognosis was determined in two ways: 1) Early poor prognosis was defined as death or neurologic sequelae due to SE within 30 days of SE as in several previous studies, and 2) Late poor prognosis was defined as death or neurologic sequelae due to SE in the late period (at the end of 13.6 ± 4.6 months of follow-up). All other conditions except death and neurologic sequelae were defined as good prognosis.

6 Statistical Analysis

The study data was analyzed using the Statistical Package for Social Sciences (SPSS) version 18, and a P value of <0.05 was considered statistically significant. Parametric (independent samples t-test and one-way ANOVA) and non-parametric tests (Mann-Whitney and Kruskal-Wallis tests) were used to compare serum levels of biomarkers in the study and control groups, and to determine their relationship with the prognosis, etiology and refractoriness of SE. Bonferroni and Dunnett were used for post hoc analysis in ANOVA. "ROC curve" was used to determine a cut-off value for sICAM-1 levels to predict poor outcomes.

When power analysis was done with 26 patients and 26 controls, the value was 0.85. We had 42 patients and 28 controls in this study.

Results

Fourty-two patients were diagnosed as having SE; 19 were males (45.2%) and 23 were females (54.8%) and their ages ranged between 17 and 90 years (mean: 50.5±18.9 years). Classification of patients based upon the SE subtype revealed GCSE in 25 patients (59.5%), EPC in 10 patients (23.8%), and NCSE in 7 patients (16.7%). Eleven patients (26.2%) were diagnosed as having RSE. Early prognosis was good in 31 (73.8%) and poor in 11 (26.2%) patients, and late prognosis was good in 20 (47.6%) and poor in 22 (52.4%) patients. One patient with NCSE and another patient with GCSE developed neurologic sequela directly related with SE. All other patients with poor outcomes died of underlying etiologies and their complications.

Detailed analysis of etiologic factors established that the underlying cause of SE was tumors in 9 patients (21.4%); infectious/metabolic/toxic causes in 10 (23.8%), including 3 patients with encephalitis (7.1%); discontinuation of anti-epileptic drugs in 4 (9.5%); genetic causes in 3 (7.1%); sequelae (operated tumors, head trauma) in 3 (7.1%) patients. The less common etiologic factors were as follows: Multiple sclerosis, anoxia, hippocampal sclerosis, neuronal migration abnormality and cerebral hemorrhage due to leukemia and thrombocytopenia in 1 patient (2.4%) for each. The etiologic factor could not be determined in 8 patients (19%). The demographics, clinical features and etiologies of the patients are presented in Table 1.

Levels of serum sP-selectin and sICAM-1 were compared between the SE and control groups, in subgroups of SE (GCSE, NCSE and EPC) and the control group and only in subgroups of SE. Also levels of these molecules were compared in different etiologies.There were 28 subjects in the control group, which consisted of 12 men (42.8%) and

16 women (57.2%) with a mean age of 43±13.5 years. The control and study groups were sex-matched. The mean age of the control group was lower than the study group without a statistically significant difference (P: 0.075).

Levels of serum sP-selectin and sICAM-1 were higher in the SE group compared with the control group with P values of 0.04 and 0.02, respectively (Table 2). It was shown that higher levels of serum sICAM-1 correlated with late poor prognosis, and "ROC curve" analysis showed that levels higher than 457 ng/ml predicted poor outcome with 71% sensitivity and 68% specificity. The area under the curve was 0.721 and P value was 0.017 (Figure 1). Also, high serum levels of sICAM-1 slightly correlated with early poor prognosis (P: 0.047). There was no correlation between levels of sICAM-1 and refractoriness of SE. No correlation was established between levels of sP-selectin and the prognosis or refractoriness of SE (Table 3).

Subgroup analyses revealed levels of serum sICAM-1 were significantly higher in EPC group compared with the control group (P: 0.012). In subgroup analyses, other comparisons for sICAM-1 (GCSE vs control, and NCSE vs control) and all comparisons for sP-selectin (GCSE vs control, NCSE vs control and EPC vs control) revealed no differences. No correlation was established between levels of sICAM-1 with the prognosis and refractoriness of SE in the EPC group, but levels of sICAM-1 were higher in patients whose SE lasted more than 6 hours (n=5) compared with those whose SE lasted less than 6 hours (n=5) (P: 0.011). Minimum SE duration was 90 minutes and maximum was 2 months in patients with EPC. Serum levels of sP-selectin and sICAM-1 in the SE subgroups and controls are presented in Table 4. Bonferroni and Dunnett tests for post hoc analysis in ANOVA for multiple comparisons of sICAM-1 are shown in table 5.

We could not find relationship between poor outcome in the short and long period and refractoriness (P values were 0.1 and 0.3, respectively). Comparisons of sICAM-1 and sP-selectin according to SE groups (GCSE, NCSE and EPC) revealed no differences. The etiologies of the patients were very heteregeneous in this study and we categorized the etiologies to better distinguish those patients with poor outcome where biomarkers gave some clues. First, we made two groups: 1- Patients with brain tumors (n=9) 2- Others (n=33). There was no difference between groups in terms of sICAM-1 and sP-selectin levels (p: 0.76 and 0.37, respectively) in Mann-Whitney Test. Also there was no difference between patients with brain tumors+poor outcome (n=6) and patients with other etiologies+poor outcome (n=16) in terms of sICAM-1 and sP-selectin levels (p: 0.23 and 1, respectively) in Mann-Whitney Test. Second, we made 5 groups: 1-Brain tumors (n=9), 2- Infections (n=5) (CNS or systemic), 3-Metabolic-toxic causes (n=5), 4- Unknown cause (n=11) (We included patients with genetic etiology to this group), 5- Other causes (n=12) (MS, hipocampal sclerosis, bleeding due to thrombocytopenia, double cortex, sequela of trauma, anoxia and operated epidermoid cyst in brain and AED discontinuation (These 4 patients were using AEDs due to prior meningitis, stroke, lesions that were not clear whether they were tumors or caused by candida and possible

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autoimmune encephalitis)). There was no difference between groups in terms of sICAM-1 and sP-selectin levels (p: 0.63 and 0.92, respectively) in Kruskal-Wallis Analysis. Also there was no difference between patients with brain tumors+poor outcome (n=5), infections+poor outcome (n=4), metabolic-toxic causes+poor outcome (n=4), unknown cause+poor outcome (n=5), other causes+poor outcome (n=3) in terms of sICAM-1 and sP-selectin levels (p: 0.49 and 0.86, respectively) in Kruskal-Wallis Analysis.

It was previously shown that serum levels of adhesion molecules were not age dependent in the 18-65 years age range (28) but there is no data for people aged more than 65 years in the literature.

Therefore, we compared the levels of serum sP-selectin and sICAM-1 between patients aged more than 65 years (n=11) and those aged less than 65 years. There was no statistical important difference between groups for sICAM-1 (P: 0.56) and sP-selectin (P: 0.27).

Table 1: Demographics, clinical features and etiologies of the patients

Demographics
    Males (n=19, 45.2%)
    Females (n=23, 54.8%)
    Mean age: 50.5± 18.9 years
SE types
    GCSE (n=25, 59.5%)
    EPC (n=10, 23.8%)
    NCSE (n=7, 16.7%)
Refractory SE (n=11, 26.2%)
Early prognosis
    Good (n=31, 73.8%)
    Poor (n=11, 26.2%)
Late prognosis
    Good (n=20, 47.6%)
    Poor (n=22, 52.4%)
Etiologies (n=42)
    Tumors (n=9, 21.4%)
    CNS infection (n=3, 7.1%)
    Systemic infection (n=2, 4.8%)
    Metabolic-toxic (n=4, 9.5%)
    Metabolic disorder and infection   (n=1, 2.4%)
    Multiple sclerosis   (n=1, 2.4%)
    Sequelae (operated tumors, head trauma) (n=3, 7.1%)
    Genetic   (n=3, 7.1%)
    Unknown   (n=8, 19%)
    Anoxia   (n=1, 2.4%)
    Cerebral hemorrhage due to leukemia and thrombocytopenia (n=1, 2.4%)
    Discontinuation of AED   (n=4, 9.5%)
    Neuronal migration abnormality   (n=1, 2.4%)
    HS (n=1, 2.4%)

CNS: Central nervous system, AED: Antiepileptic drug, HS: Hippocampal sclerosis, SE: Status epilepticus, GCSE: Generalized convulsive status epilepticus, EPC: Epilepsia partialis continua, NCSE: Non-convulsive status epilepticus

Table 2: Levels of serum sP-selectin and sICAM-1 in SE and control groups and P values.

	SE	Control	P
sICAM-1 (ng/ml)	479.1 ± 168.9 (N=40)	392.4 ± 109.1 (N=28)	**0.020**
sP-selectin (ng/ml)	163.8 ± 63.8 (N=42)	133.4 ± 47.7 (N=26)	**0.040**

Table 3: Levels of serum sP-selectin and sICAM-1 in SE group according to early and late prognosis and refractoriness and their P values.

	Early good prognosis	Early poor prognosis	p
sICAM-1 (ng/ml)	448.7 ± 154.5	570.6 ± 185.2	**0.047**
sP-selectin (ng/ml)	158.4 ± 60.8	179.2 ± 72.5	0.360
	Late good prognosis	**Late poor prognosis**	**p**
sICAM-1 (ng/ml)	410 ± 139	542 ± 172.1	**0.017**
sP-selectin (ng/ml)	159.6 ± 53.5	167.7 ± 73	0.685
	Non-refractory SE	**Refractory SE**	**p**
sICAM-1 (ng/ml)	469.6 ± 160.3	507.7 ± 198.9	0.544
sP-selectin (ng/ml)	162.4 ± 66.4	167.7 ± 58.6	0.816

Table 4: In the table, median, minimum and maximum values (on the first line); mean and standard deviation values (on the second line) of serum levels of molecules are shown. The superscripts a, b, c and d show the subgroups EPC, JCSE, NCSE and controls respectively with the asterisks notation *: $P < 0.05$ show the significance level of the multiple comparisons of posthoc tests.

Molecules	EPC	GCSE	NCSE	Controls	ANOVA
sICAM-1 (ng/ml)	521.5 (229-931) 551.7 ± 214.5 [d*]	433 (217-706) 440.2 ± 147.6	487 (362-787) 503.6 ± 145.5	374 (217-627) 392.4 ± 109.1 [a*]	P<0.05
sP-selectin (ng/ml)	171 (70.4-230.1) 160.2 ± 49	172.7 (11.7-316.3) 168 ± 69	150.4 (87.7-290.1) 154.3 ± 70.8	137.2 (42.8-256.8) 133.3 ± 47.7	P: 0.212

Table 5: Bonferroni and Dunnett tests were used for post hoc analysis in ANOVA for multiple comparisons of sICAM-1.

Multiple Comparisons
Dependent Variable:sICAM-1

	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Bonferroni	JCSE	EPC	-111,526	54,845	,277	-260,85	37,80
		NCSE	-63,398	62,501	1,000	-233,57	106,78
		Healthy subjects	47,781	40,746	1,000	-63,16	158,72
	EPC	JCSE	111,526	54,845	,277	-37,80	260,85
		NCSE	48,129	71,354	1,000	-146,15	242,41
		Healthy subjects	159,307*	53,340	,024	14,08	304,54
	NCSE	JCSE	63,398	62,501	1,000	-106,78	233,57
		EPC	-48,129	71,354	1,000	-242,41	146,15
		Healthy subjects	111,179	61,185	,443	-55,41	277,77
	Healthy subjects	JCSE	-47,781	40,746	1,000	-158,72	63,16
		EPC	-159,307*	53,340	,024	-304,54	-14,08
		NCSE	-111,179	61,185	,443	-277,77	55,41
Dunnett t (2-sided)[a]	JCSE	Healthy subjects	47,781	40,746	,546	-51,45	147,01
	EPC	Healthy subjects	159,307*	53,340	,012	29,40	289,21
	NCSE	Healthy subjects	111,179	61,185	,195	-37,83	260,19

*. The mean difference is significant at the 0.05 level.

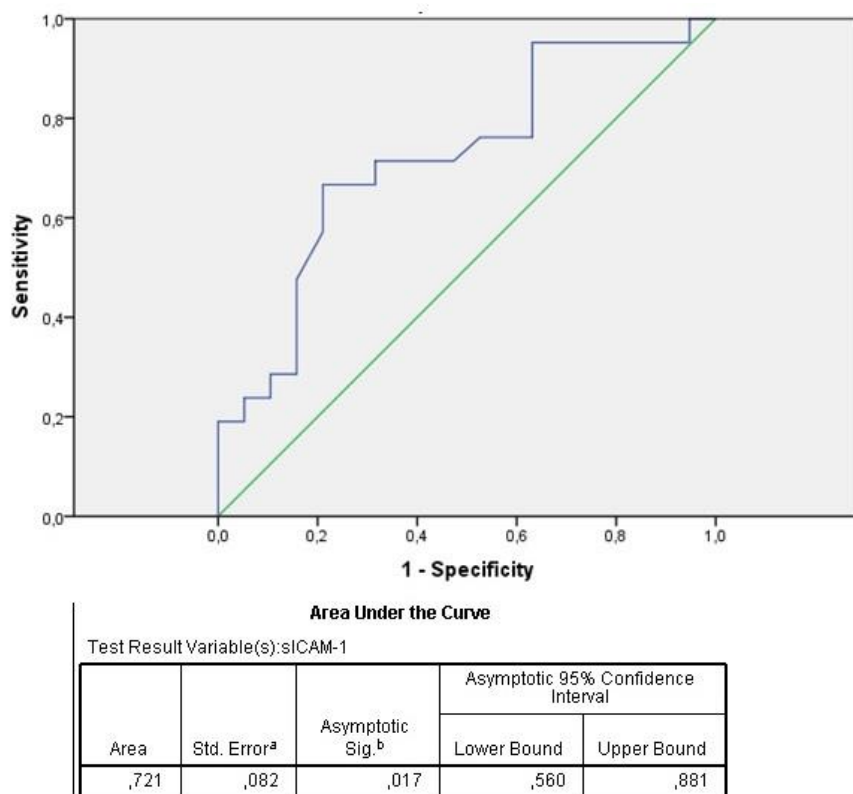a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Area Under the Curve

Test Result Variable(s):sICAM-1

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
,721	,082	,017	,560	,881

Figure 1: "ROC curve" analysis of serum levels of sICAM-1 predicting poor outcome in status epilepticus. AUC (area under the curve): 0.721, P: 0.017.

Discussion

Our study determined that serum levels of sICAM-1 were higher in the EPC group compared with the controls and higher serum levels of sICAM-1 predicted poor outcome in SE patients; therefore, we concluded that it could be used as a biomarker of SE in clinical practice. However, serum levels of sICAM-1 and sP-selectin did not change according to either SE types (comparisons in GCSE, NCSE and EPC groups, excluding controls) or etiology.

Refractoriness is expected to correlate with poor outcome. However, in our recently published study, which used univariate analyses, refractory SE was related with poor outcome in the short period (p: 0.013) but unrelated in the long period (p: 0.114) and also lost its significance in the short period when multivariate analyses were performed (33). In this study, we could not find any relationship between poor outcome in the short and long period and refractoriness. Six of our 7 patients with cryptogenic etiology had refractory SE which could cause less worse outcome in patients with refractory SE because patients with unknown etiology may have good outcomes although they have refractory SE. On the other hand, the small size of our patient group could have contributed to this result.

An extensive literature search highlighted the lack of studies that measured serum levels of sICAM-1 and sP-selectin in patients with SE. A human study compared tissue samples obtained from patients with hippocampal sclerosis (HS) during epilepsy surgery with brain tissue samples obtained from patients who died of non-neurologic

causes during autopsy. The authors demonstrated CD4 and CD8 positive lymphocytes and diffuse ICAM-1 staining in the hippocampal parenchyma in patients with HS but no lymphocytes and only a weak ICAM-1 staining in the control group (24). A recently published study reported that levels of serum sICAM-1, and serum and CSF sVCAM-1 were higher in patients with drug-resistant epilepsy compared with patients with drug-responsive epilepsy and patients with newly-diagnosed epilepsy, which suggested that these molecules could have an important role in drug-resistant epilepsy (27). Additionally, another study demonstrated that CSF levels of neuronal adhesion molecule-1 (NCAM-1) were lower in patients with drug-resistant epilepsy compared with patients who were drug-responsive, and in both patient groups compared with controls (25). It has also been established that serum levels of anti-inflammatory sICAM-5 were found lower in patients with drug resistant epilepsy (26). The relation between epilepsy and inflammation has been established in a study which indicates that a cascade of inflammatory process are initiated following an epileptogenic event at birth or later in life that contributes to the development of epilepsy and recurrent seizures (34). At a molecular level, seizures induce a series of inflammations in brain endothelial cells leading to an upregulation of IL-1β and its receptor IL-1R1 (35), the complement system (36), and adhesion molecules (P-selectin, E-selectin, ICAM-1 and VCAM-1) (21, 22).

Another result obtained from this study was that serum level of sICAM-1 in particular increases in EPC, which suggests that sICAM-1 may play a role in the pathogenesis of EPC. Although inflammation is known to have an important role in the pathogenesis of the EPC subtype associated with Rasmussen encephalitis (37) which is also related with anti-GluR3 (38) antibodies, several pathologies of the motor strip might also cause EPC and there are no conclusive reports of the relationship between EPC and sICAM-1. EPC is a rare type of focal status epilepticus. Distinct pathologies can cause EPC such as inflammatory disorders 32%, neoplastic disorders 19%, head trauma 16%, vascular disorders 14%, others 3% and unknown 16% which were reported in a review (39). In our study, the following etiologies were observed in the 10 patients with EPC: tumors (n=5), central nervous system infection (n=1), systemic infection and metabolic disorders (n=2), and an etiology that could not be determined (n=2). Although we found increased sICAM-1 levels in the EPC group, we did not find a relationship between levels of sICAM-1 and different etiologies of EPC. This may be due to small number of patients. Also, the production of sICAM-1 may increase as a common pathway, regardless of etiology in EPC. On the other hand, levels of sICAM-1 were higher in patients with EPC whose SE lasted longer than 6 hours in our study which may suggest that production of sICAM-1 increases in time in EPC. No correlation was established between levels of sICAM-1 with the prognosis and refractoriness of SE in the EPC group but the sample size of this group was too small for these analyses and studies with larger number of patients are needed.

Our study had limitations, one was the small sample size, which did not allow for determination of an association between sICAM-1 and sP-selectin serum levels with SE etiology. Another problem was that these serum levels could only be measured once, thus repeat measurements were not taken to determine whether the levels returned to normal after the SE event. Also, measurements were not performed at the most appropriate time periods as 6, 12, or 24 hours after the start of SE. Obtaining these measurements during SE might lead to a false negative result. We did not check for atherosclerotic risk factors in patients, but we mostly excluded patients with atherosclerotic risk factors by excluding those in whom the etiology was cerebrovascular disease. One could also argue that a molecule that is affected by atherosclerotic risk factors is not a good choice for determining the prognosis of SE.

A recently-published study by Trinka et al. (29) proposed 2 time points for SE. The first time point (t1) is the time beyond which the seizure should be regarded as "continuous seizure activity." The second time point (t2) is the time of ongoing seizure activity after which there is a risk of long-term consequences including neuronal injury. In the case of convulsive SE, t1 at 5 min and t2 at 30 min was proposed, and t1 at 10 min and t2 at >60 min were suggested in cases of focal SE with impaired consciousness. Patients who had a generalized convulsive seizure longer than 5 minutes were diagnosed as having GCSE and were included in our study. Of the 44 patients

included, 37 had GCSE longer than 30 min, which is likely to cause neuronal injury according to Trinka et al., but seven of our patients with GCSE had a seizure duration shorter than 30 minutes.

A study performed on rats demonstrated that vascular endothelial cells started ICAM-1 expression 6 hours after intraventricular injection of kainic acid, followed by vascular accumulation and migration of lymphocyte function-associated antigen-1 (LFA-1) positive leukocytes in the hippocampal region (23). Another study on mice revealed that both the production and leukocyte adhesion of ICAM-1, VCAM-1, E-selectin, and P-selectin were increased following pilocarpine-induced seizures or SE. Significant reduction in SE and spontaneous seizures were observed upon treatment of mice with monoclonal antibodies specific to these molecules and granulocyte-specific antibodies (22). A study on humans, as mentioned before, indicates that sVCAM-1 and sICAM-1 could play an important role in the drug-refractory epilepsy (27).

Conclusion

This is the first study conducted on human subjects to demonstrate the value of serum levels of sICAM-1 and sP-selectin in the prediction of the course and prognosis of status epilepticus (SE). Investigation of these biomarkers on higher number of patients and the determination of the connection between their high levels and outcome of the SE would give us the chance to choose more aggressive antiepileptic drug treatment and to reduce morbidity and mortality in patients with SE. The results of our study and animal studies suggest that the production of sICAM-1 may be increased as a common pathway, regardless of etiology in SE and especially in EPC. However, further studies are required to elucidate the specific role of sICAM-1 and sP-selectin in the pathogenesis of SE, to investigate the relationship between etiology and SE and to consider these molecules as potential targets in SE treatment; because predicting outcome is usually not enough for clinicians and the biomarkers are expected to provide information to develop novel approaches in terms of treatment of the condition.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Effect and reliability of transcranial magnetic stimulation on neuropathic pain in stroke patients: Preliminary study

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Abstract

Objective: In this paper, we aimed to evaluate the effect of repetitive Transcranial Magnetic Stimulation (rTMS) on central pain in patients with stroke.

Material and Methods: Ten patients who had a stroke history were included in this preliminary study. Patients with middle cerebral artery lesion, first-time stroke, subacute or chronic lesion and no other neurological involvement were included. Patients were examined before and after the first month of treatment with repetitive Transcranial Magnetic Stimulation (rTMS). Visual Analog Scale (VAS) was used for pain scale measurement; Leeds Assessment of Neuropathic Symptoms and Signs Scale (LANSS) and Douleur Neuropathique en 4 Questions (DN4) were used for neuropathic pain level and Beck Depression Inventory (BDI) for depressive mood. All patients received the current treatments and appropriate neurorehabilitation as recommended for the treatment of ischemic stroke

Results: This prospective study included a total of 10 post-stroke patients (mean age 58.2 ± 16.1 years; range 29 to 75, 8 male; 2 female) with neuropathic pain. The mean values of VAS, LANSS, DN4 and BDI scales were significantly decreased after rTMS treatment in all patients.

Conclusion: We discussed the preliminary results of the efficacy and safety of rTMS in the treatment of uncontrolled neuropathic pain. We consider that, rTMS may have significant effect on relief of chronic pain. These clinical parameters can be utilized for the further study of rTMS application in pain control.

Key words: Transcranial magnetic stimulation, stroke, pain.

Introduction

Stroke is a major cause of death and disability. After stroke, survivors have typically neurological squeal and stroke-related complications. The duration of stroke rehabilitation depends on the severity of stroke and related complications. Pain is one of the most common complications in patients with stroke and may adversely affect patients' quality of life (1). Opioids, tricyclic antidepressants or anticonvulsants have been found to be useful for the treatment of neuropathic pain in stroke patients (2). Despite various clinical trials there is still no consensus about the best strategies for the management of neuropathic pain (3). The other safer and non-invasive way to provide analgesic neurostimulation is also Transcranial Magnetic Stimulation (TMS).

TMS was approved by the US Food and Drug Administration for the treatment of major depression in 2008, and researchers are still investigating TMS for a number of other neurological conditions, including chronic and neuropathic pain. Studies have reported that TMS techniques are effective in central pain and may provide pain relief by altering the cortical excitability (4-6).

The prevalence of central post stroke pain was reported as 7.3% after stroke (7). Quality of life (QoL) decreases by 40% compared to pre-stroke at the end of one year after stroke (8). It can play an important role on quality of life, mood and rehabilitation as it is known that pain affects recreational activities, occupational status and sleep quality.

For most of the CPSP patients, pain is eventually associated with a hard and reduced physical function, reducing quality of life. Moreover, pain and psychological disorders are closely related, affecting their physical and psychosocial functioning. Likewise, CPSP has a significant effect on QoL and a strong relationship between pain and depression has been observed (9).

The aim of this study was to investigate the effect of TMS on neuropathic pain in a small number of subjects as a preliminary report.

Material and Methods

This prospective study included a total of 10 post-stroke patients with neuropathic pain. Age, gender, hemiplegic side and duration of stroke were investigated.

The study protocol was approved by the Gaziantep University Clinical Research Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki. The inclusion criteria were as follows; middle cerebral artery lesion, first-time stroke, subacute or chronic lesion and no other neurological involvement. The exclusion criteria were as follows; multiple lesions on cranial imaging, aphasia, head trauma, acute stroke, epilepsy in their medical history. Pregnant women were also excluded. Table 1. shows an overview of patients based on demographic characteristics in this study. A written informed consent was obtained from each participant. All patients were evaluated with a full neurologic examination for sensory disorder, motor deficits, and spasticity.

Patients were evaluated at baseline and after the first month of treatment in respect to all clinical parameters.

Pain intensity was evaluated by a 10 cm Visual Analog Scala (VAS) asking for the pain experienced during the last week (10). This scale is 10 cm length. Slight pain is considered to be VAS<3.3, moderate pain 3.3-6.6, severe pain VAS 6.7-9.9 and unbearable pain VAS 10. We used two questionnaires for the diagnosis of neuropathic pain; Leeds Assessment of Neuropathic Symptoms and Signs Scale (LANSS) and Douleur Neuropathique en 4 Questions (DN4). LANSS has high sensitivity and specificity for identifying neuropathic pain. The LANSS pain scale was first used by Bennett to clinically distinguish neuropathic pain from nociceptive pain. Shortness of application time and ease of evaluation are the advantages of this test. The reliability and validity of the pain scale of LANSS was performed in 2004 by Yücel A. et al. (11). LANSS contains five symptom and two clinical examination items. The first part consist five items asking the patient about the kind of pain experienced in the last week. In the second part, presence of allodynia and determination of pinprick perception threshold are explored by health care professional. Each item should be marked as present or absent, and the presence of each sign has different score. The possible scores range from 0 to 24, with a score of 12 or greater considered to be suggestive of neuropathic pain.

DN4 is a questionnaire for neuropathic pain consisting of interview questions and physical tests. A score of 1 is given to each positive item and a score of 0 to each negative item.

The total score is calculated as the sum of the 10 items, and a total score of 4 or more out of 10 suggests neuropathic pain (12).

We assessed post stroke depression by the Beck Depression Inventory (BDI), this method being used by other studies. BDI score over 30 was associated with a severe depression (13,14).

All patients received a rehabilitation program according to each participant's functional level or requirements. Range-of-motion exercises were applied passive, active-assistive, or active, strengthening exercise, flexibility, balance and coordination training also applied in all stroke patients.

In each TMS session, 30 trains of 10 Hz stimuli for a duration of 5 seconds at an inter-train interval of 25 seconds, a total of 1500 pulses, was applied. Total duration of a TMS session was 15 minutes at intensity equal to 80% of the resting motor threshold. This area was identified as the site at which single pulse TMS contra-laterally evoked a motor potential of maximal amplitude in the first dorsal interosseus muscle of the hand, ipsilateral to the painful zone. This procedure ensured stimulation over the precentral gyrus.

The patients received the TMS, while sitting in a comfortable chair or their wheelchair. A total of 10 sessions of TMS treatment was performed in 5 sessions per week. The levels of current, duration, and stimulation types were performed by the same physiotherapist. Side effects were reported by means of this physiotherapist after each stimulations.

Statistical Analysis

The analysis were performed with SPSS (Statistical Package for Social Sciences) 22.0 program. Paired sample's T test was used to compare the clinical parameters before and after treatment. Significance was evaluated as $p < 0.05$.

Results

The gender distribution was as 8 males and 2 females. The mean age was 58.2 ±16.1 years, mean disease duration was 2.5 ±1.7 years. All of the patients had chronic pain. The mean duration of pain was 2.0±0.7 years. Pain was localized at lower limbs at hemiplegic side and the other body parts. All patients had first ischemic cerebrovascular etiologies. Hemiplegia was on the right side in 6 patients (60%) and on the left side in 4 patients (40%). When comparing to mean difference of scores from baseline to following treatment, there was significant improvement in all clinical parameters. The mean scores of VAS, LANNS, DN4 and BDI scales were significantly better at first month of treatment in compared to baseline. TMS could improve either pain or depressive mood in all patients after the treatment sessions. Furthermore, no side effects were observed during TMS treatment. The characteristics of clinical parameters in stroke patients are determined in table 2.

Table 1. Demographic features in stroke patients

Duration of disease (year)	1 (min)	7 (max)	2.5±1.7 (mean ± SD)
Affected side	6 (right)	4 (left)	60/40 (ratio)
Age	29 (min)	75 (max)	58.2±16.1
Sex	8 (male)	2 (female)	80/20 (ratio)

SD: standart deviation; min: minimum; max: maximum

Table 2. Comparison of clinical parameters in stroke patients

	Before treatment (Mean±SD)	After treatment (Mean±SD)	*P*
LANSS	9.4±4.4	6.0±3.2	*<0.001**
VAS	4.8±1.4	3.7±1.4	*<0.001**
DN4	4.4±2.8	2.9±2.3	*0.001*
BDI	17.2±6.9	13.6±5.9	*<0.001**

LANSS: Leeds Assessment of Neuropathic Symptoms and Signs Scale; VAS: Visual Analog Scala; DN4: Douleur Neuropathique en 4 Questions; BDI: Beck Depression Inventory; *$p < 0.05$: statistically significant.

Discussion

Pain after stroke can significantly affect functionality and may adversely affect the physical activity of patients, however, there is a little data on this topic. The onset of pain in our cases occurred 1 to 4 months after the stroke. The presentation of the CPSP is variable. Adjectives such as lacerating, aching, burning, freezing, and squeezing are commonly used by patients, as observed in our patients (15). The neuropathic pain most commonly begins 1 to 6 months after stroke (16).

In some case central pain diagnosis is delayed due to the fact that patients are not hospitalized in rehabilitation clinics, their cognitive status is impaired and some of them are aphasic. It is also claimed that neuropathic pain exhibits a latent period which may be up to 18 months after stroke onset (17). In stroke survivors, the emotional status may be altered because of the functional and cognitive consequences of stroke and neuropathic pain. We evaluated 10 patients with a previous history of chronic pain who were resistant to other modalities followed in our rehabilitation clinic.

Neuropathic pain is sometimes resistant to current pharmacological treatments. Further, there are several side effects of analgesic drugs such as dizziness, drowsiness (18). In the last decade, the effects of TMS on excitatory and inhibitory cortical circuits have been used in patients with chronic pain.

Our current study showed that the neuropathic pain making difficult to treat could possibly minimize with applying TMS in patients with post stroke. We have also seen that, TMS can potentially offer a non-invasive treatment option for neuropathic pain in patients with stroke.

The analgesic effects of TMS in chronic pain have been investigated and it has been shown to provide analgesic effects in chronic pain in recent years (19-22).

Interestingly, we found a significant reduction in depression level, immediately after a rTMS sessions in patients with stroke.

Studies have shown that motor cortex stimulation has inhibitory effects on thalamic and spinal nociceptive neurons (23). Neuroimaging studies have shown that epidural electrical stimulation of the premotor cortex in patients with neuropathic pain increases blood flow in distant brain areas (eg, lateral and medial thalamus, anterior cingulate cortex, insula, brain stem) (24). It has been demonstrated that rTMS given to motor cortex activates the endogenous opioid system in a wide brain network associated with processing of pain (25).

It is unknown any procedure should be perform whether rTMS therapy is more effective in the acute or chronic stage after stroke. In our study, all patients had subacute or chronic lesion.

Stimulation frequency is play a major role on the analgesic efficacy of stimulation. It has emphasized a short-term effect of a single stimulation session of high-frequency rTMS directed at the motor cortex (26). The authors concluded that this method was not suggestive of a beneficial treatment effect for chronic pain patients long-term follow-up (27). In an another study, Hosomi et al. (28) reported data on neuropathic pain patients treated with rTMS to premotor cortex for 10 days. But they obtained a significant short term pain relief not lasting after the sessions. Short et al. (29) have shown a marginal analgesic effect of rTMS up to 2 weeks after the stimulation phase, using parameters similar to those of our study (10 Hz).

Studies have been reported that TMS was improved the neuropathic pain, with minimal side effects (30). Most safety issues concern the effects of magnetic fields on the human body, especially the risk of seizures during sessions. In our study no patients had side effects during the stimulation phase or at follow-up. We aimed that targeting

the premotor cortex in an induction 10 repeated sessions would provide an analgesic effect in pain with stroke patients (31).

According to our results, all patients tolerated well with high-frequency TMS (10 Hz) and demonstrated appreciable pain relief benefit without any side effects.

The current study has several limitations. This is a relatively small preliminary study that lacks a control group, we have shown significant benefits from rTMS for patients with neuropathic pain syndromes. Another study limitation is that the long-term effects of TMS treatment were not investigated and the data were not objective.

Conclusion

TMS is a non-invasive, short-lasting, painless and focal way modality that modulates cortical excitability. Although much has learned about the central pain modulatory mechanisms about TMS, very little is known about its mechanisms and efficacy in modulating neuropathic pain conditions. In literature controlled prospective studies are rare and the technique is still not largely used. The optimal timing for long-term efficacy and safety of post-stroke pain are still unknown. Further studies should be explored the effects of different parameters and long-term stability. Randomized controlled studies are required in further validating the efficacy of this treatment modality. Additional, studies are required to assess the underlying mechanisms of analgesia.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: TT, MSA, NT, ÖA: Research concept and design; data collecting, TT, MSA, NT, ÖA ŞŞ: Preparation of article, and Revisions. All authors approved the final version of the manuscript

Ethical issues: All Authors declare, originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities.

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Fine needle aspiration cytology (FNAC) of cystic soft tissue lesions and end tissue metamorphosis-a three year study

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Abstract

Objective: Superficial soft-tissue masses may be seen in clinical practice, but a systematic approach may help to achieve a definitive diagnosis or differential diagnosis for soft tissue lesions. The cystic lesions constitute a heterogeneous group with highly varied etiology, cytology and diversified histopathology. The aim of this study is to investigate the accuracy of FNAC diagnosis of varied cystic lesions of soft tissue lesions by comparing with the radiological and histopathology diagnosis.

Materials and Methods: Fine needle aspirations were done using a 22-24 gauge disposable needle and a 5cc to 10 cc disposable syringe for suction. Wet-fixed smears with isopropyl alcohol were stained with hematoxylin and eosin (HxE). Dry-fixed smears were stained with Leishman Giemsa along with Papanicolaou stains (PAP) were studied for cytological details and diagnosis. The excised surgical specimen and biopsy samples of the cases were processed routinely and stained with HxE and immunohistochemistry (IHC) panel was applied.

Results: Examined cystic soft-tissue masses were found as superficial (82%) and deep (18%). Superficial lesions were categorized into mesenchymal tumors, skin appendage lesions, tumor like lesions, pseudoudo tumoural soft tissue lesions or parasitic /inflammatory lesions. Deeper lesions with cystic presentation were mostly (74%) malignant. The differential diagnosis was done according to the age of the patient, anatomic location of the lesion, salient imaging features and clinical manifestations.

Conclusion: Although the fine needle aspiration cytology of the cystic lesions, imaging characteristics of the lesions discussed are not always corresponding to the histopathologic findings what we assume, combining them with lesion location and clinical features may allow the diagnosticians to suggest a specific diagnosis in most cases.

Keywords: FNAC, Cystic soft tissue lesions

Introduction

Cystic Soft tissue tumors are defined as nonepithelial extraskeletal tissue of the body exclusive of the reticuloendothelial system, glia, and supporting tissue of various parenchymal organs (1, 2). The absence of recognizable tissue architectural patterns in cytological preparation and in cases of inflammation, rupture and trauma makes diagnosis by FNAC more difficult. Soft tissue lesions or soft tissue tumor like cases may deceive pathologist and radiologist. This soft tissue structures known as oops lesions which are focused in this study may defined according to following the final histopathological diagnosis.

The histological subtyping of soft tissue tumors in fine-needle aspiration (FNA) smears is more reliable when dealing with well differentiated tumors showing specific cytomorphological diagnostic and immunocytochemical criteria. Conversely, poorly differentiated soft tissue sarcomas can represent a difficult diagnostic group because of lack of distinctive morphological and immunocytochemical criteria. Ancillary techniques are used in and out nowadays as diagnostic help in morphological diagnosis of soft tissue lesions. Essentially the same ancillary techniques are used for cytological as for histopathological diagnosis like after preparing cell blocks.

The most commonly used technique is Immunocytochemistry. In addition cytogenetic and molecular biological techniques as well as electron microscopy play an important role. Techniques such as polymerase chain reaction (PCR) and fluorescence in situ hybridization have been proved to be suitable for fine needle aspiration samples (1,3,4). The technique for FNA for soft tissue tumours is the same as for other types of lesions. A good guided aspiration is essential for accurate diagnosis. Frequent problems occurring can be due to missing of the lesion altogether by aspirator and reactive changes mimicking malignant cystic sarcomas (3,4). In addition, representative diagnostic areas may be difficult to aspirate from cystic, necrotic or haemorrhagic masses

Materials and Methods

The aim of the study was to know the accuracy of Fine needle aspiration cytology for diagnosis of varied cystic lesions of soft tissue in correlation with ultrasonography and histopathology diagnosis. Aspirations were done using a 22-24-gauge disposable needle and a 5cc to 10cc disposable syringe for suction. Wet-fixed smears with isopropyl alcohol were stained with hematoxylin and eosin (HxE). Dry-fixed smears were stained with Leishman Giemsa along with PAP Stain were studied for cytological details and diagnosis. The excised surgical specimen and biopsies of the cases were processed routinely and stained with Hematoxylin and Eosin and IHC panel was applied wherever requirement was there.

Statistical Analyzes

Percent of Mean and SD values of the stains were compared and presented in the tables.

Results

Out of total 60 patients accounted for 53 lesions were benign and 7 were malignant. The 41 out of 53 benign lesions were truly cystic lesions and 12 were partly cystic and solid. All the 7 malignant lesions were partly cystic and solid. The 82% of the total lesions were superficial and 8% were deep seated. Table 1 shows the distribution and types of benign cystic lesions, table 2 points to partly cystic lesions and table 3 deals with malignant cystic lesions. Statististical analysis showed that the sensitivity of FNAC for malignant lesions was 75% and for benign lesions 97.5% (Table 4,5). Sensitivity, specificity followed by positive predictive value and negative predictive value were analyzed for both benign and malignant cystic soft tissue lesions (Table 5).

Benign cystic lesions were anechoic semi cystic to cystic lesions (Fig1-4).Partially cystic lesions had mixed echogenicity comprising of anechoic to hyperechoic regions (Fig 5). Malignant cystic lesions were mostly homogenous with cystic degeneration and increased vascularity (Fig6-7). Occurrence of oops lesion was also seen (Fig 8-9).

Table1: Classification of studied benign cystic lesion.

Type of lesion	Age	Male	Female	Location	Usg finding	Total
Ganglion cyst	20-30	5	2	Finger<foot<ankle<wrist	Thick walled anechoic lesion	07
Synovial cyst	40-50	1	1	Joints<tendon	Anechoic lesion,thick wall	02
Bursal cyst	All ages	1	2	Knee<elbow<pop fossa	Thick wall,anechoic lesion	03
Epidermal inclusion cyst	All ages	8	7	Forearm<face<neck<trunk	Thick wall,fine intermission	15
Cystcercosis	40-50	2	1	Thigh<pubic area	Anechoic lesion,cystic spaces	03
Lymphocoele	20-30	2	-	Neck	Multiple thin septa,<3 mm	02
Seroma	60-70	1	1	Thigh<forearm	Anechoic,cystic mural nodule	02
Post traumatic hematomas	30-40	4	1	Lower extremity<upper extremity<abd wall	Thick irregular valves,internal breakdown	05
Cutaneous microfilaria	20-30	-	01	Subcutaneous nodule	Distended lymphatics,anechoic lesion	01
Foreign body granuloma	20-30	01	0	Sacral region	Isoechoic lesion	01
Total		25	16			41

Table 2: Classification of studied partly cystic and partly solid lesions

Type of lesion	Age	Male	Female	Location	Total
Hidradenitis suppurata	30-40	0	1	Axilla	1
Glomus tumours	20-30	1	2	Thumb, ankle	3
Lymphangioma	20-30	1	0	Suboccipital	1
Hemangioma	<20	1	0	Face	1
Myxoma	40-50	0	1	Thigh	1
Schwannoma	40-50,	1	1	Gluteal Region, Leg	2
Neurofibroma	50-60	2		Thigh ,Shoulder	2
Giant cell tendon	20-30	1		Sheath tumour	1
Total		7	5		12

Table 3: Clasification of studied cystic malignant lesions:

Lesion	Age	Male	Female	Location	Usg finding	Total
Synovial sarcoma	40-50	1	-	Gluteal region	homogeneously hypoechoic subcutaneous cystic mass	1
Myxoid liposarcoma	40-60	1	1	Thigh	heterogeneous echoic mass without internal hypervascularity	2
Cystic malignant nerve sheath tumour	50-60	-	1	Thigh	Heterogenous hyperechoic mass	1
Myxofibrosarcoma	40-50	1		Abdominal wall	Homogenous hypoechoic mass	1
Inflammatory MFH	30-40	01		Elbow	Complex hypoechoic mass with increased vascularity	1
Undifferentiated pleomorphic sarcoma with intense inflammation and cystic degeneration	70-80		1	Gluteal region	homogeneous echo pattern and hypoechoic margins with cystic changes and internal vascularity	1
Total		3	4			7

Table 4: Number of benign and malignant lesions cases.

	Benign Cyst	Malignant	Total
Benign	39(true negative)	2(false negative)	41
Malignant	1(false positive)	6(true positive)	7

Table 5: Statistical comparison of USG sensitivity of benign and malignant lesion cases

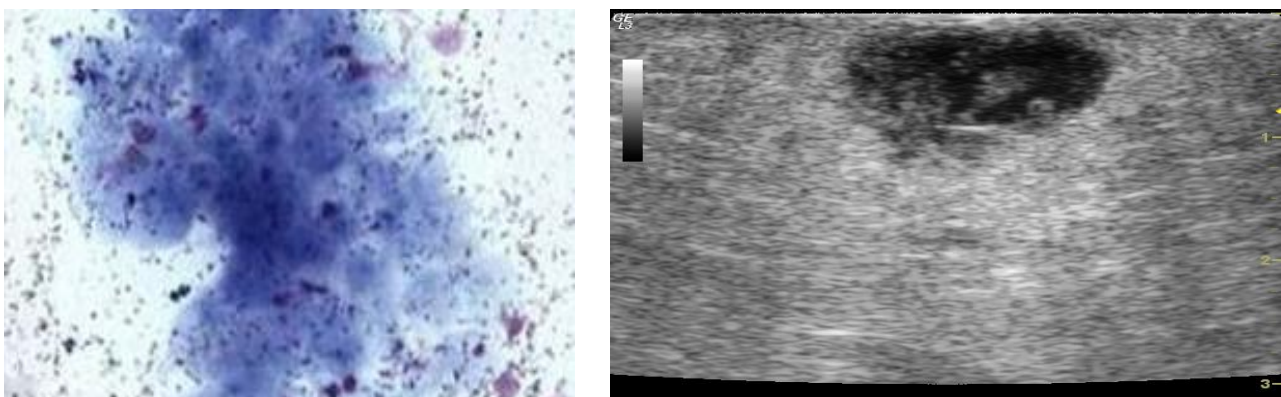| USG sensitivity for cystic malignant lesions	True Positive(TP)/TP+FalseNegative(FN)	75%
Specificity of Benign cystic lesions	True negative(TN)/TN+FN	97.5%
Positive predictive value of malignant lesions	TP/TP+FN	75%
Negative predictive value of benign lesions	True negative/TN+FN	90%

Figure 1: Cysticercosis cellulose with cyst wall in FNAC and rupture of the cystic wall on sonography.
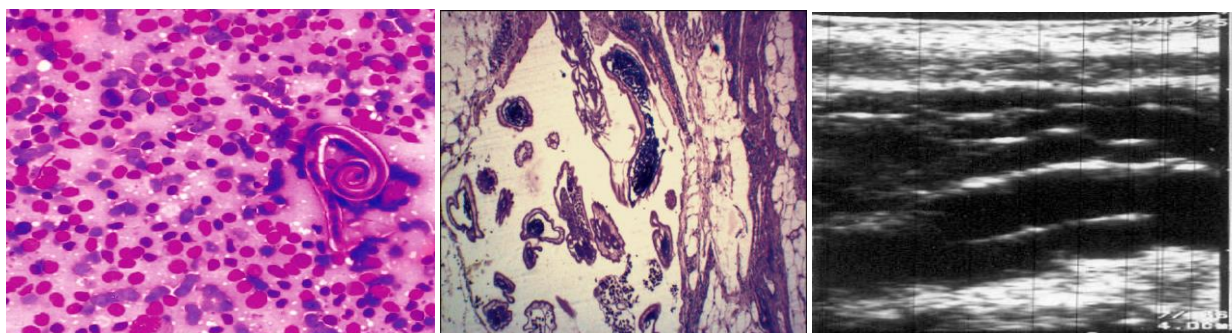
Figure 2: FNAC, HPE, USG: microfilarial worm, filarial worms were noted , lymphatics distended lymphatics in sonography in axilla-microfilaria on FNAC cystic presentation.
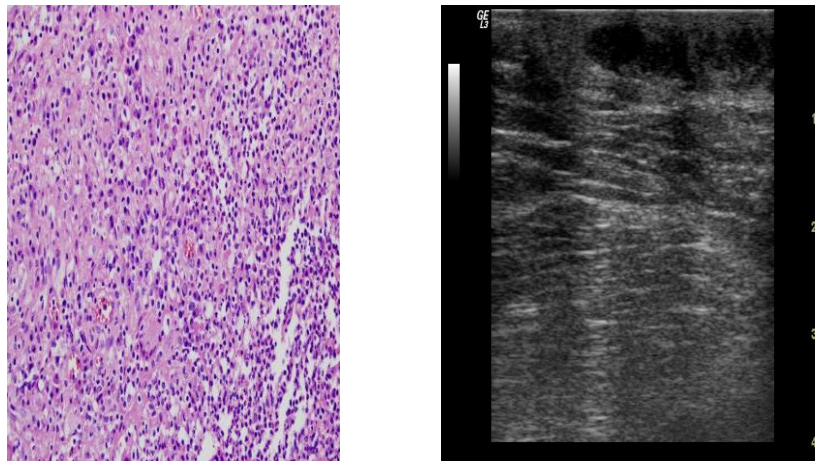
Figure 3: Lymphocoele with cystic presentation in an inflammory background with anechoicto isoechoic presentation with thin septa
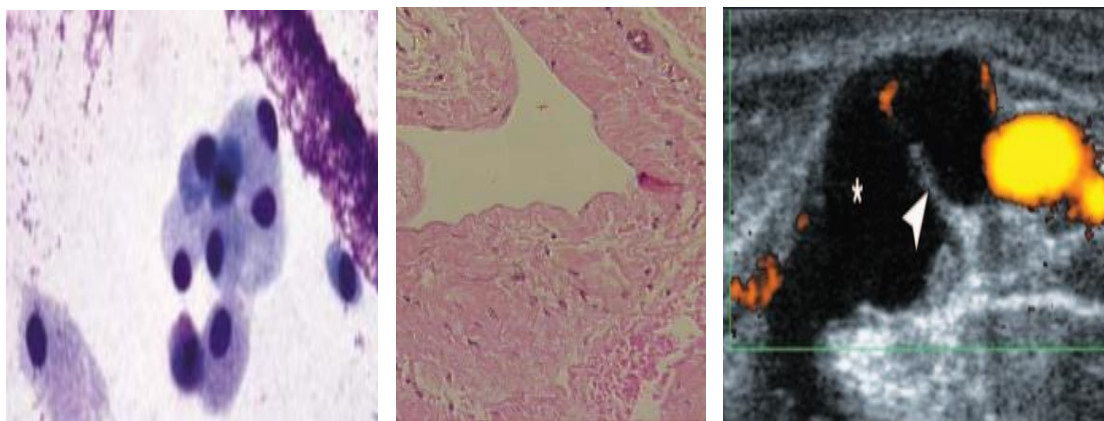
Figure 4: FNAC from ganglion cyst shows a few histiocytic like cells with pale abundant cytoplasm, myxoid background and USG shows anechoic center, thin septa (arrowhead) without increased vascularity on Doppler sonography.
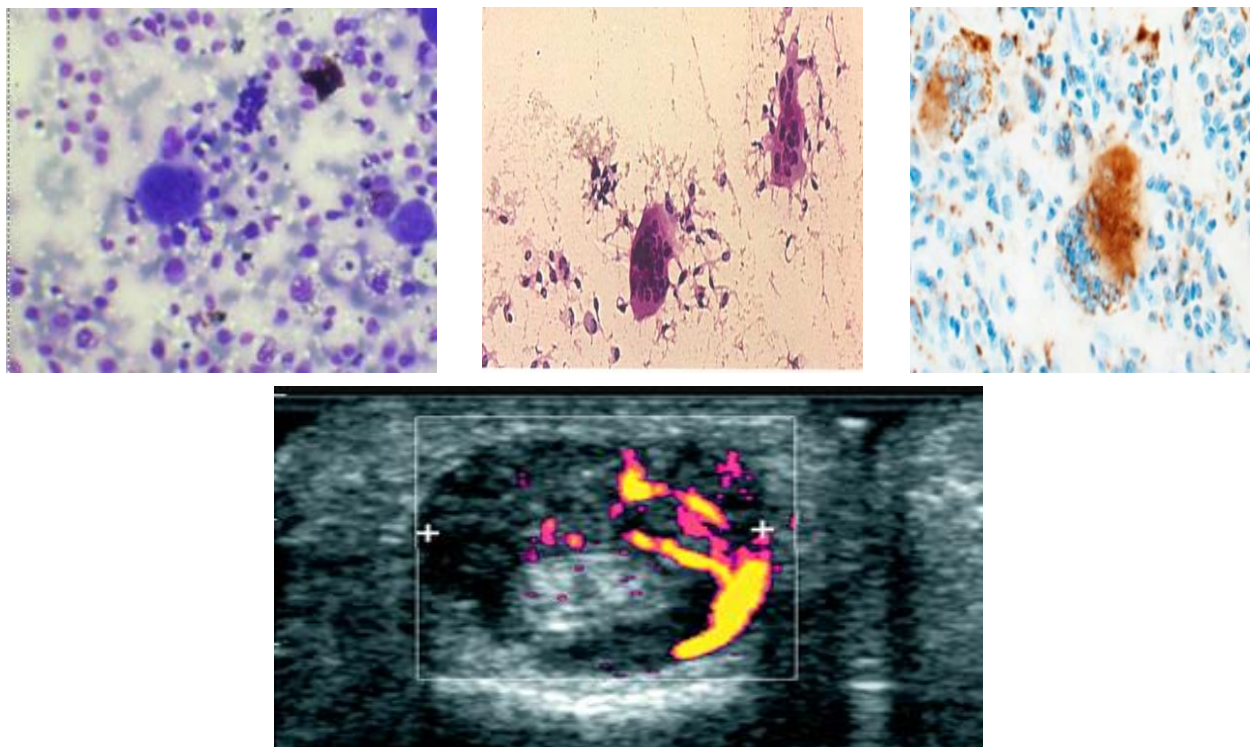
Figure 5: Giant cell tumour of tendon sheath female, index finger. FNAC shows loose clusters of spindle cells and a variable number of multinucleated giant cells with pigment (CD 68 positivity for giant cells). Transverse color power Doppler sonogram shows that tumor (*cursors*) has readily detectable blood flow located peripherally and centrally.

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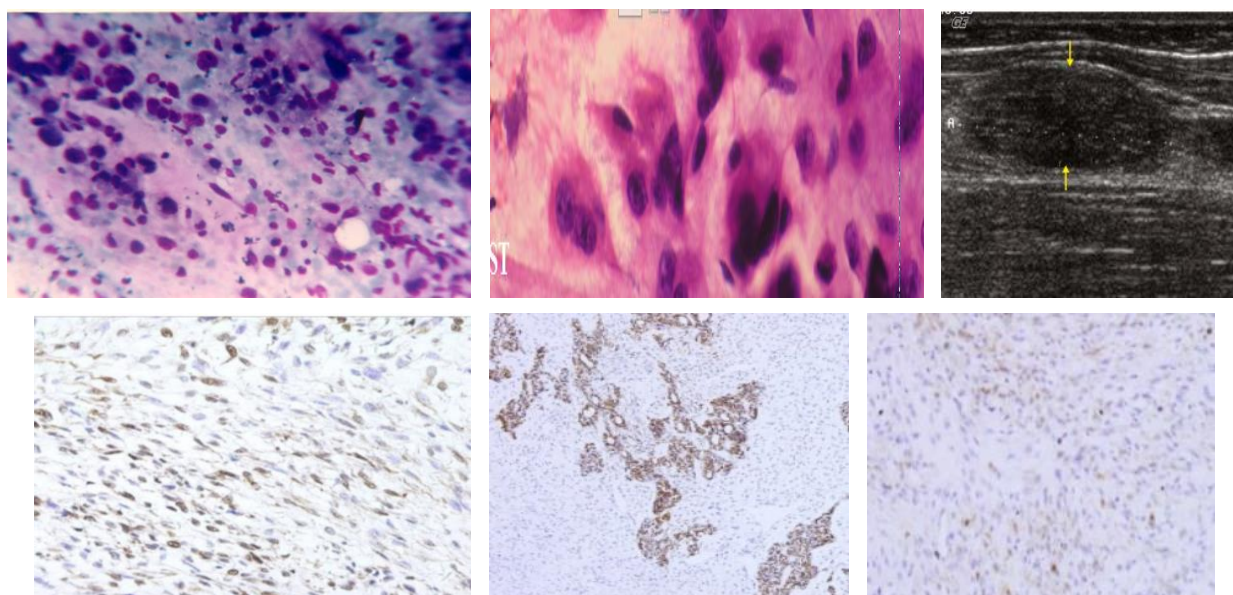
Figure 6: Malignant peripheral nerve sheath tumor (Mpnst). Cystic soft tissue swelling over forearm in 30 yr old male. Cohesive clusters and single spindle cell or pleomorphic pattern. USG shows heterogeneous hypoechoic nodule (arrows) in the left forearm with marginal infiltration. Respective spindle cell positivity for S100, Cyatokeratin and Vimentin.
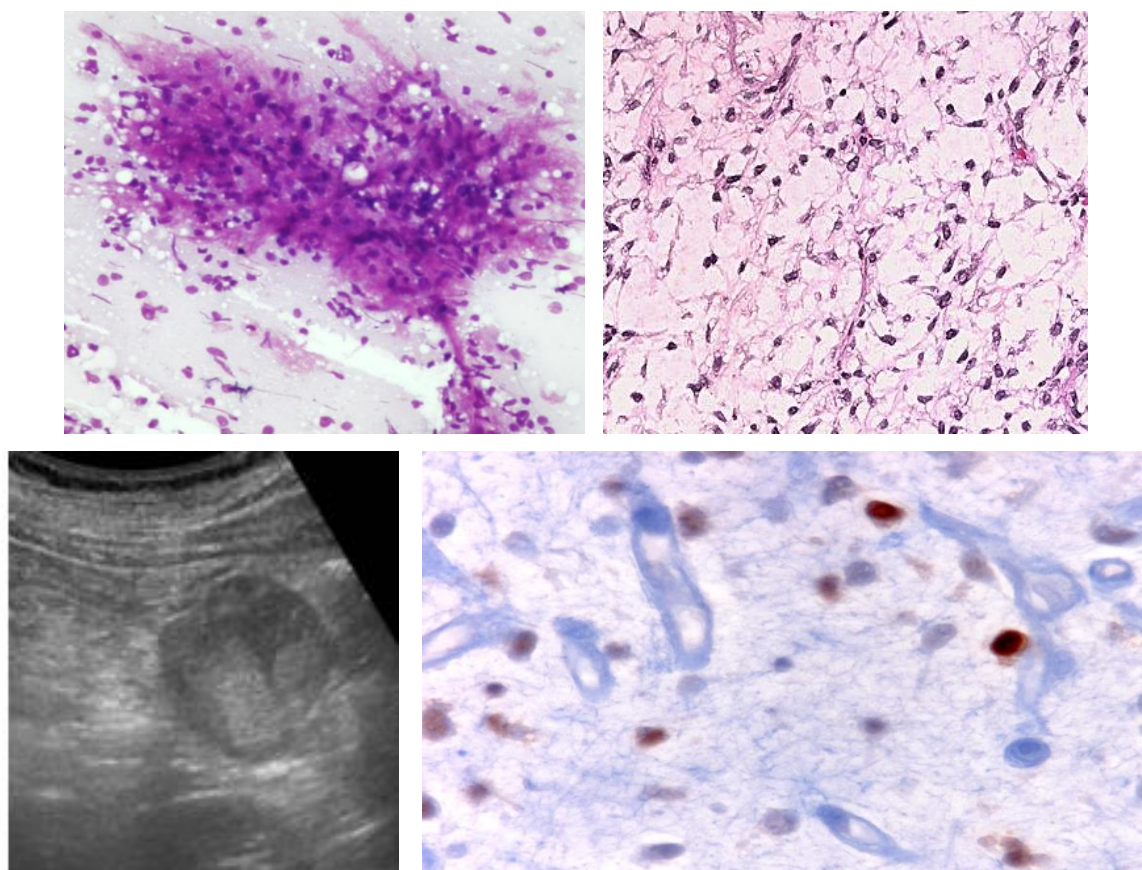
Figure 7: Myxoid stroma with plexiform capillary network,Numerous vacuolated cells. Cells have round to ovoid nuclei ,usg shows heterogeneous echoic mass without internal hypervascularity as evidenced in the color Doppler ultrasonography with S100 positivity on Immunohistochemistry
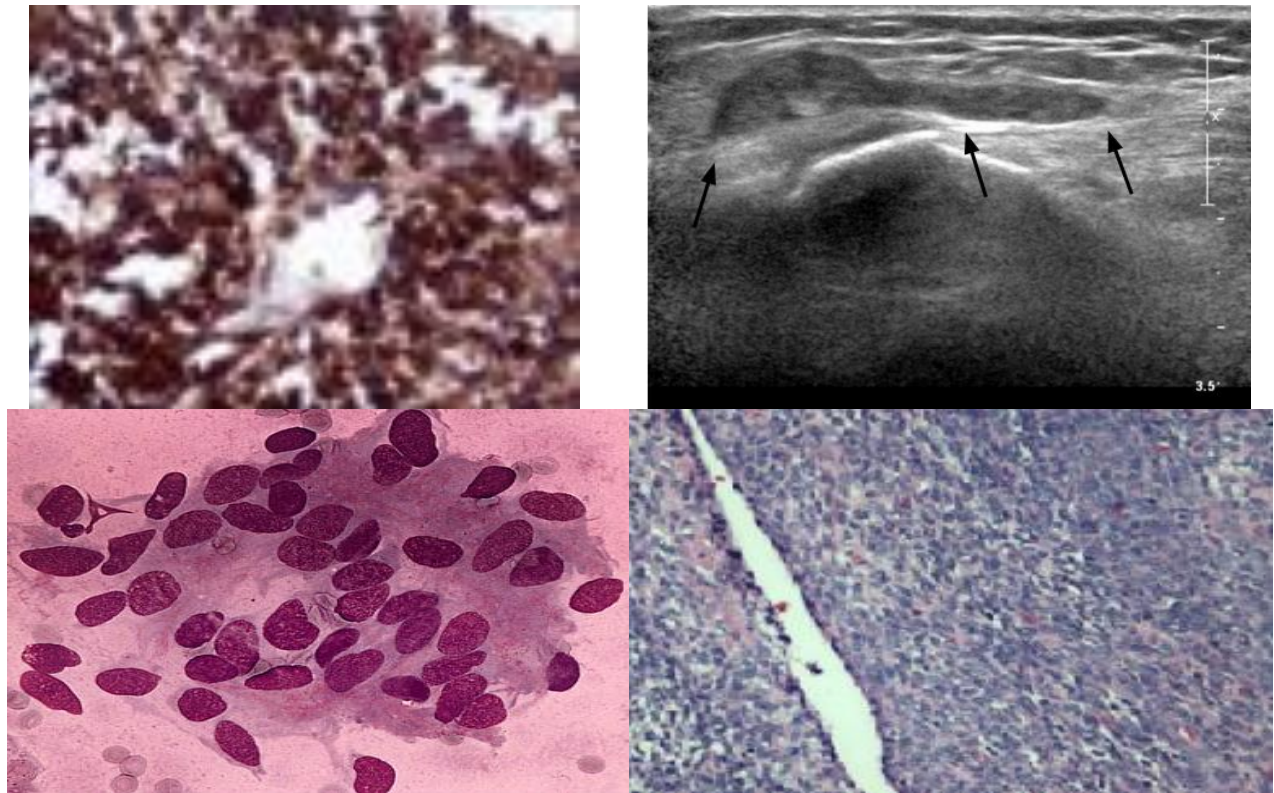
Figure 8: OOPS LESIONS, Benign Looking Malignant Lesions: Elderly female, Ultrasonography shows a homogeneously hypoechoic subcutaneous cystic mass in the lateral aspect of the left knee joint. Benign lesion was suggested.Cluster of oval to spindle cell noticed, given as malignant spindle cell lesion. Histology and IHC with cytokeratin positivity, confirmed the tumours to be cystic biphasic synovial sarcoma

Figure 9: Malignant looking benign lesions: Ultrasonography elderly, female, shows a well-defined heterogeneously hyperechoic mass on the abdominal wall. At the corner of the mass biopsy a needle is seen. Nonneoplastic tissue with hemorrhage, fibrin exudation, and hyalinization was observed. Cystic cavity with necrotic debris, fibrin and blood clots on HPE.

Discussion

Fine needle aspiration cytology is proved to be useful and convenient method in diagnosis of cystic soft tissue tumors. It gives fairly accurate diagnosis when combined with clinical and USG findings (3,4). The concept of combining Diagnostic cytology and therapeutic surgery for soft tissue sarcoma was based on assumptions that since open biopsy is omitted contamination by tumor cells in the wound hematoma is avoided and surgical margins can be left less extensive with minor predictable loss of function (5,6). However, observed cystic changes in the tumours can hinder maliciously the real findings of the same.

There are not many published series which extensively discuss FNAC of cystic soft tissue tumors with sonographic studies (7,8,9). USG though is not gold standard but remains the most commonly used diagnostic modality due to portability, cost effectiveness and lesser time taken (12-14) .The present study showed that USG has 95% accuracy in cases of malignant tumors. Hence FNAC is a safe, useful screening test with good diagnostic accuracy when supported by other diagnostic data (9-11).

The present study, whose aim was to prove the efficacy of FNAC as a useful tool and a reliable technique in diagnosing soft tissue tumors, showed a sensitivity of 81.2%, specificity of 95.75%, positive predictive value of 81%, and negative predictive value of 92% that well correlated with other studies. Therefore, when a differential diagnosis for a cystic soft tissue mass is made, all available information should be considered, such as demographic data, laboratory findings, and findings from other imaging modalities (15-19). In addition, practitioners should be familiar with the variability in US imaging findings (20-23).

Conclusion

Fine needle aspiration cytology of cystic soft tissue tumours has important limitations. Samples may be limited in cellularity as a result insufficient for a diagnosis. There are certain neoplasms in which a benign versus malignant differentiation cannot be made with certainty from FNAC and it may be impossible to predict the grade on the basis of the cytology smears especially with spindle cell neoplasms that can be difficult in a background of cystic changes.US findings are helpful in the diagnosis of soft tissue tumors (24,25,26). However, the diagnosis of soft tissue tumours on the basis of US findings alone has some limitations. Thus, it is not unexpected that "oops lesions" are encountered during clinical practice but still from the above observations, we can conclude that FNAC of STT has several advantages that outweigh disadvantages. It is a useful, safe, cost-effective and easily performed as outpatient procedure for the evaluation of soft tissue tumors with accuracy rate at par with that of biopsy. It provides a rapid, non-traumatic procedure for sampling both superficial and deep seated mass lesion. Multiple samples can also be obtained during a single clinical visit thereby increases the likelihood of specimen adequacy for other ancillary tests. Our study proves the efficacy of FNAC in the diagnosis of soft tissue tumors as a useful cost–effective procedure as the results showed that the diagnostic accuracy of FNAC of soft tissue tumors is 95.37. In the light of all these results, the necessity of the use of differential methods beside FNAC for soft tissue lesions detection and classification should not be ignored.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: RT, RSB, PG: Research concept and design, Patient examination and Sample/data collecting, **RT:** Preparation of article and Revisions. All authors approved the final version of the manuscript

Ethical issues: All Authors declare, originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities.

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The relationship between illness perception and quality of life in thyroid patients who received radioactive iodine-131 ablation treatment

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Abstract

Objective: Most of the differentiated thyroid carcinoma (DTC) patients recover totally after the primary treatment. Unlike most of the other cancer types, the patients do not go through a long, challenging and weary treatment process in thyroid cancers. Illness perception has been suggested to have a significant effect on quality of life on cancer patients. We aimed to evaluate the effect of illness perception on quality of life in thyroid cancer patients who had radioactive iodine-131 treatment after total thyroidectomy.

Methods: Totally one hundred patients were included in this cross-sectional study. Patients' age, educational level, marital status and disease characteristics including stage, treatment and follow-up period since diagnosis were reviewed. Short Form 36 Health Survey Questionnaire (SF-36), the illness Perception Questionnaire were used to assess the quality of life and illness perception, respectively.

Results: When we correlated the illness perception sub-scales and quality of life dimensions in our study, the most apparent negative correlation was seen between the immunity dimension of the disease sub-scale and quality of life subdimensions. There was also a negative and significant (p<0.05) correlation between the dimensions of ability to understand the disease and overall health.

Conclusion: The low ability of patients to understand the disease significantly affects the negative impact on the quality of life of the disease perception. The needs of patients monitored during the remission period should be determined and a multidisciplinary approach should be preferred when necessary.

Keywords: Thyroid, Ablation, Quality of Life, Perception of Disease, Radioactive Iodine-131

Introduction

Thyroid cancer is the most common tumor on the endocrine gland. Well-differentiated thyroid carcinoma for which radioactive iodine-131 (RAI-131) treatment is administered accounts for about 85-90% of all thyroid cancers. The rate of psychiatric disorders in cancer patients is high. The meanings ascribed by patients to the cancer, and their way of perceiving the disease affect the response to cancer and the quality of life (1). Besides, medical, psychological and social factors play an important role in this response process. The determinants of the psychological response in patients diagnosed with cancer include variables such as type of cancer, treatment methods, adverse effects of the treatment, psychological maturity (2, 3, 4).

The World Health Organization defines the "Quality of Life" as "an individual's perception of their position in life in the context of the culture and value systems in which they live in relation to their goals, expectations, standards and concerns" (5). Quality of life decreases in chronic diseases and mental disorders (6). Investigations made on how the disease is perceived shows that illness perception affects individual's emotional reactions, strength of coping with problems, and accordingly, the quality of life. Illness perception is a dynamic process that involves people's beliefs about their diseases, and their cognitive views of the disease.

An individual interprets the internal and external stimuli that might be associated with the disease in his/her mind, and creates an image of threat by attributing a meaning to them. Cognitive processes resulting from such stimuli mediate the development of disease representations (7).

In this cross-sectional study, we aimed to determine the potential impact of "illness perception" on quality of life scores in well differentiated thyroid cancer patients who had received radioactive iodine 131 treatment after total thyroidectomy.

Materials and Methods

Ethic

This study was conducted at a single center in Gaziantep University. The protocol and informed consent documentation were reviewed and approved by the Independent Ethics Committee of the University and agreed with the ethical principles of the Declaration of Helsinki.

Patient selection

A total of 100 thyroid cancer patients who were treated in Gaziantep University Nuclear Medicine Department between December 2015 and December 2016 were recruited for this crosssectional study. We assessed well differentiated type thyroid cancer patients who had received radioactive iodine 131 treatment after total thyroidectomy.

The 'inclusion criterias' were as follows:

1. 18 years old or older when diagnosed with well differentiated type thyroid cancer diagnosed histopathologically

2. Having total thyroidectomy surgery

3. Received radioactive iodine 131 treatment

4. Established euthyroid state with hormone replacement therapy

The 'exclusion criterias' were as follows:

1. Combination with another tumor

2. Major morbidity, such as chronic obstructive pulmonary disease, coronary heart disease, cerebrovascular disease

3. Subclinical hyperthyroidism/hypothyroidsm, Overt hyperthyroidism/ hypothyroidism

Data Collection

Participants completed a standardized questionnaire assessing the demographic factors and medical data, illness perception and quality of life. Thus, the following measures were used:

Short form 36 (SF36) Health Survey Questionnaire:

SF-36 Quality of Life Scale was developed by Sullivan et al (1995), and its Turkish validation-reliability study was conducted by Koçyiğit et al (1999) (8,9). This scale evaluates 8 different subsets of health.

The items in SF-36 not only question the positive situations but also the negative ones about health. Scores of the items are coded for each dimension, and turned into a scale that is scored from 0 (the worst health state) to 100 (the best health state).

Illness perception Scale:

The Illness Perception Scale (IPS) was developed by Weinmann et al (1996), and reviewed by Moss-Morris et al (2003) (10,11). The Turkish interpretation of the scale and the validation-reliability study were performed by Armay et al (2007) on cancer patients (12). A reviewed IPS form was used in the study. IPS includes the dimensions of disease type, views on the disease, and causes of the disease.

Statistical Analysis

All data obtained from the study has been analyzed by using the SPSS 15.0 statistics program. After completion of the definitive statistical analyses, compliance of the variables to the normal distribution was evaluated by means of the Shapiro-Wilk Test. Fischer's Exact test, Yates' Chi-square test and Pearson's Chi-square test were used to determine whether the groups differ in terms of discrete variables; Student t test, and Mann-Whitney U test were used in binary comparisons of continuous variables, when parametric assumptions were met, and were not met, respectively. Furthermore, illness perception sub-dimensions and quality of life sub-dimensions were compared in our study using the RHO correlation test of Spearman.

Results

Patients social demographic charecterisitics were shown on the table 1. A total of 100 well differentiated thyroid cancer patients who had received radioactive iodine 131 treatment after total thyroidectomy with stage I-IV disease were involved in this study (Each stage has 25 patients e.g. 25 patients for stage I). The mean age of patients enrolled in the study was 40.62 ± 10.28. There were 84 (84.0%) female and 16 (16.0%) male cases. Mean laboratory and clinical features values of the patients were shown in Table 2.

When we compared the illness perception sub-scale dimensions and quality of life sub-dimensions, we observed a negative and significant ($p<0.05$) correlation between the dimension of ability to understand the disease under views on disease in illness perception scale and overall health dimension under quality of life scale; between emotional representations dimension and vitality and overall health dimensions under quality of life scale; between the sub-dimension of immunity under the causes of the disease dimension and all sub-dimensions under quality of life scale, between psychological references and mental health dimension, and between accident and chance dimension and the mental health dimension under the quality of life scale (Table 3).

There was a significant difference between the Outcomes dimension of views on the disease sub-scale under illness perception of married and single cases (p=0.035). The Outcomes dimension scores of the married cases were significantly higher than those of single cases ($p<0.05$).

There was not a significant difference between age and illness perception scores of cases ($p>0.05$).

Table 1: Socialdemographiccharecteristics of theindividuals

Factors	Variables	Number	%
Gender	Female	84	84,0
	Male	16	16,0
EducationalStatus	None	18	18,0
	Elementary	48	48,0
	High School	24	24,0
	University	10	10,0
MaritialStatus	Married	86	86,0
	Single	14	14,0
Total		100	100,0

Table 2: Mean laboratory and clinical features values of the patients

Parameter	Patients	Standart Deviation
Age (year)	40,62	10,28
BMI (kg/m^2)	31,75	0,5
TSH (mU/l)	2,67	0,20
free-T3 (pmol/l)	3,56	0,1
free-T4 (pmol/l)	1,05	0,4
Thyrogloblin	1,09	0,2
Anti-Thyrogloblin	4,67	0,24
Duration of thedisaese (Month)	5,63	0,5

Table 3: Results of the correlation analysis of the relationship between the illness perception and quality of life.

		1	2	3	4	5	6	7	8
Disease Type		-0.22	-0.23	-0.31*	-0.27	-0.30	0.03	-0.21	-0.39*
Views on the Disease	Time	-0.21	-0.22	-0.18	-0.12	0.08	-0.16	-0.05	-0.14
	Conclusions	-0.29	-0.18	-0.15	-0.40*	-0.31	-0.18	-0.25	-0.32*
	Personal Control	0.10	-0.03	-0.03	-0.11	0.02	-0.06	0.04	-0.08
	Treatment Control	-0.24	-0.10	-0.01	-0.06	0.01	-0.05	0.04	0.37*
	Ability to Understand the Disease	0.00	-0.34*	-0.27	-0.26	-0.24	-0.11	-0.08	-0.42**
	Time (Cyclic)	-0.38*	-0.32*	-0.21	-0.23	-0.24	-0.32*	-0.29	-0.07
	Emotional Representations	-0.28	-0.24	-0.15	-0.42**	-0.38*	-0.28	-0.37*	-0.31*
Causes of the Disease	Psychological References	-0.34*	-0.20	-0.21	-0.31	-0.48**	-0.16	-0.34	-0.27
	Risk Factors	-0.29	-0.22	-0.19	-0.35*	-0.36*	-0.46**	-0.10	-0.24
	Immunity	-0.45**	-0.40**	-0.35**	-0.42**	-0.37**	-0.46**	-0.24	-0.32*
	Accident or luck	-0.29	-0.31	-0.23	-0.33*	-0.47**	-0.36*	-0.26	-0.35*

1- Physical Function 2- Physical Role Limitations 3- Emotional Role Limitations 4- Vitality 5-Mental Health 6-Social Function 7- Pain 8- Overall Health Perception *$p<0.05$, **$p<0.01$

Discussion

Most of the DTC patients recover totally after the primary treatment. Unlike most of the other cancer types, the patients do not go through a long, challenging and weary treatment process in thyroid cancers. However, unexpected developments that are beyond our perception might be seen in patients during the disease process, and the quality of life might fall. Even though there are a few studies that evaluate the quality of life of DTC patients, none cover its relationship with the illness perception [13, 14].

The main approach in our study has been the illness perception, and the extent to which such perception affects the quality of life. In a study, the only demographic factor that was found to be effective on the illness perception was education [15]. Besides, the number of iodine treatments was found to have a significantly negative effect on the illness perception. In our study, the patient group that had repetitive treatment was excluded.

None of the demographic factors was concluded to be significantly effective on the illness perception.

In the first phase of our study, a general assessment was made on the disease type, views about the disease, and causes of the disease sub-scale dimensions of the illness perception scale. In the disease type sub-scale dimension of our study, patients were asked if they experienced the 14 symptoms since the onset of disease, and whether these symptoms were associated with the disease. The high rate of the 'yes' responses given to the second question shows the severity of disease symptoms. The most common symptom was found to be fatigue also in a study conducted by Zordan et al [16]. This result was consistent with almost all studies found in literature on the chronic diseases. Fatigue and loss of strength symptoms were reported as the most frequent ones in almost all stages without any correlation with the treatment period of DTC patients.

The scores of time (acute/chronic) dimension were slightly high in our study. A high score in this dimension is a negative parameter showing the patient believes that his/her disease is chronic. It was seen that some of the patients thought that the disease would last long, it was permanent rather than temporary, and they would live with this disease for the rest of their lives. In a study conducted by 110 thyroid cancer patients, the score of time subscale was found to be close to that of our study [15].

The scores of Outcomes sub-scale are not as high as the scores observed in other cancer cases in our study. This might be attributed to the fact that the long and weary treatment process observed in other cancer cases is not seen in DTC patients, or the psychological trauma caused by other cancer types is harder.

The mean score of 'personal control perception' and 'treatment control' dimension was high in our study. This results show that patients trust in their treatment and their own control of the treatment. High scores are positive parameters on the continuity of treatment and patient compliance. However, the overall findings show that personal control perception is low in cancer patients [1,17-20]. Treatment compliance is significantly higher in DTC patients as compared to other cancer patients.

The highest scores were observed in the 'emotional representations' dimension as a negative parameter in our study. This result shows that patients struggle with a worrisome and alarming situation. According to a study, the level of education is the only demographic factor that affects the emotional representation of disease [20]. In our study, no significant correlation was found between the level of education and emotional representations. The score of emotional representations was high in all groups. The ability to understand the disease dimension scores, on the other hand, were low in our study. The low scores in this sub-scale show that patients cannot understand the disease sufficiently, or have difficulty in understanding the disease. On the contrary, the ability to understand the disease dimension received the highest score along with the personal control in another study [15]. The only varying socio-demographic factor in that study was the level of education. The higher this factor gets, the more the ability

to understand the disease score increases and the further the Outcomes dimension including the belief that disease might have serious Outcomes decreases. In our study, no correlation was observed with the level of education. In the study conducted by Karabulutlu et al with other cancer patients in our country, the scores in the ability to understand the disease dimension were significantly lower [22].

The third sub-scale of illness perception was 'causes of the disease' in our study. Psychological references ranked first in this scale, followed by risk factors with a little difference. In a study conducted on other cancer types, risk factors were the first sub-dimension. This is caused by the belief of our people that smoking ranks the first among the risk factors of cancer [23].

The reason behind the fact that psychological references rank first might be the intense feelings of stress, distress, and family problems especially in the group with low socio-economic level, as a reflection of overall life challenges. It was found that such negative factors affected the patients positively in terms of staying away from stress during the treatment process.

The correlation between the quality of life sub-dimension and illness perception sub-dimension was examined in the next part of the study. The mean physical functions sub-scale score was 53.90 in our study. The high scores in this dimension show that the person can perform his/her daily activities without any restrictions. When correlated with the illness perception, the most significant correlation was between the disease factors and immunity sub-dimension. In cases that gave the response "low body resistance" as the cause of disease, physical function score was significantly low.

A negative and significant correlation was found between the sub-dimension of ability to understand the disease and 'overall health' dimension of the quality of life scale; between the risk factors under causes of disease and 'social function' dimension of the quality of life scale; and between 'emotional representations' sub-dimension under illness perception scale and vitality sub-dimension under quality of life scale.

Conclusion

The dimension of ability to understand the disease under the views on the illness perception subscale was notably low in our study. Based on this result, it may be concluded that healthcare professionals fall short in explaining the disease. Observation of high scores in this dimension in the studies performed in developed communities has shown that we only focus on the treatment of disease, and do not put much interest in the subjective dimension of disease, which is the one perceived by the patient.

According to the illness perception scale, the scores in the emotional representations dimension, and 'personal control' and 'treatment control' dimensions were observed as positive, and negative parameters, respectively. The scores in quality of life scale were generally low. When we correlated the illness perception sub-scales and quality of life dimensions in our study, the most apparent negative

correlation was seen between the immunity dimension of the disease sub-scale and quality of life sub-dimensions. In brief, quality of life of patients believing that their body resistance was low was significantly lower than others.

In patients followed-up during the remission period with these results, a multidisciplinary approach must be preferred when required, considering the effect of illness perception on the quality of life. It would be beneficial to try different approaches on this patient population which is increasing in prevalence over time.

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Author's Contributions: **GA, UE:** Research concept and design; data collecting, **GA, GE, UE, ES:** Preparation of article, and Revisions. All authors approved the final version of the manuscript

Ethical issues: All Authors declare, originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities.

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Application of circular statistics to life science

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Abstract

Objective: The aim of the study was to explain circular statistics and hypothesis tests with birth data. The accuracy of the statistical method used in scientific research is related to the data structure and scale type. Therefore, scale types and data structures should be well defined. It is possible to frequently come across circular data in many different scientific fields such as medicine, biology and physics. These data are usually obtained by compass or clock. Compass; the flight direction of any animal that is released, the direction of the wind or the direction of current in the ocean, clock; the birth time of infants, the time of crisis, circadian rhythms or biological rhythms can be shown as examples. Apart from the clock, such data may also be obtained by a scale that expresses a time such as day, month and year.

Material and Methods: The data related to 179 normal deliveries that took place in Yüzüncü Yıl University Medical Faculty Hospital in 2008 were used. Circular data analysis was performed using the NCSS2007 statistical package program.

Results: The times of birth of infants show a uniform distribution. No significant difference at a significance level of 5% was found between the times of birth according to gender.

Conclusion: It has been stated that circular data cannot be analyzed by the analysis methods developed for linear data due to several reasons. If circular data are analyzed by linear statistical methods, inaccurate or nonsense results usually emerge. Therefore, it was emphasized that appropriate statistical methods should be used.

Key words: Circular data analysis, circular statistics, statistics, von Mises

Introduction

The suitability of statistical methods used in scientific research is directly related to the data structure and scale type. Therefore, scale types and data structures should be well defined and known by the researchers. The angular scale, which includes circular data, is usually defined within the interval scale. It is possible to frequently come across circular data related to angular scale in many different scientific fields such as medicine, biology, geology and physics (1). These data are usually obtained by compass or clock. The direction of movement of any animal that is released, the direction of the wind or the direction of current in the ocean can be shown as examples for the data obtained by compass, while the birth time of infants, the time of crisis, circadian rhythms or biological rhythms can be shown as examples for the data obtained by clock. Apart from the clock, such data may also be obtained by a scale that expresses a time such as day, month and year (2). Circular data are shown as the points on the perimeter of unit circle with central origin or the unit vectors that combine these points with origin depending on the appropriately selected zero direction and the selection of the direction of movement.

Zero direction refers to the starting point, and the direction of movement refers to clockwise or counterclockwise. Since circular data are shown on the unit circle, these observations correspond to any θo angle between 0o and 360o. θo angle is the angle between the unit vector and the starting point according to the reference direction (3).

Furthermore, the absence of a natural ordering of observations in circular data, overlapping of start and end points (0=2π), and the fact that the value θ is periodical with θ+p(2π) value for any p integer significantly differentiate circular data analysis from univariate and multivariate linear statistical analysis. Even though the need for measurements related to the selection of arbitrary zero direction and direction of movement does not make most of the classical linear statistical techniques and measurements nonsense, these techniques and measurements give incorrect results in many cases. In this context, the selection of appropriate statistical methods for circular data is important. In this study, circular data analysis methods were applied to the data on the times of birth of infants, and the interpretation of results and the suitability of these methods were discussed (4).
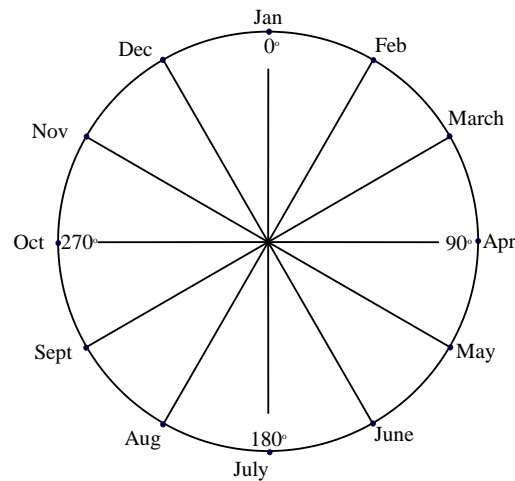
Figure 1: Circular representation of the months

Material and Methods

The research material consisted of the data related to 179 normal singleton births that took place in Van Yüzüncü Yıl University Faculty of Medicine Obstetrics and Gynecology Department between 16/04/2008 and 31/05/2008. The data set was recorded as the time of birth and baby's gender. 104 and 75 of 179 births were male and female, respectively. While all data were discussed for a single sample while evaluating the times of birth, the baby's gender (1: Male, 2: Female) was taken into account for two samples. For circular data analysis, the starting direction and the direction of rotation were taken as north and clockwise, respectively.

Whether there was any difference between the times of birth during birth events and whether there was any difference between the times of birth during birth events according to gender were evaluated by the circular data analysis method, and NCSS 2007 statistical package program was used for the evaluations (5).

Circular Descriptive Statistics: The graphical representation for getting an idea about circular data, and for the analysis and interpretation of data is one of the important stages. Different graphics and histograms are used in the representation of circular data. These graphics are used to summarize the data set and to get an idea about the data distribution before the statistical calculations.

While any point p_i in the plane is shown as (x,y) according to cartesian coordinates, it is shown as (r, θ) according to polar coordinates. Since the relationship between the directions is examined in the analysis of circular data, the vectoral sizes of data points are of no importance, and it is assumed that these data points are distributed on the perimeter of the unit circle due to the ease of operations. Thus, the distance of any point p_i to origin is 1.

Conversions are frequently performed between coordinate systems in the circular data analysis. These conversions are performed using *sine* and *cosine* trigonometric functions as the following (3).

$$cos\theta = \frac{x}{r} , \qquad sin\theta = \frac{y}{r} \qquad\qquad (1)$$

Furthermore, the data in the original scale is converted by equation (2). Here; θ gives the angular value of the data, a gives the data on the original scale and k gives the entire cycle on the scale where a is measured.

$$\theta = \frac{360.a}{k} \qquad\qquad (2)$$

Mean Direction: In the calculation of mean direction for a data set indicating concentration towards a direction, firstly, the mean components of the unit vectors p_i (i= 1, 2, ..., n) relative to the vertical coordinate system on the perimeter of unit circle corresponding to the angle θ_i are taken as the following,

$$\bar{x} = \frac{1}{n} \sum_{i=1}^{n} cos\theta_i , \qquad \bar{y} = \frac{1}{n}\sum_{i=1}^{n} sin\theta_i \qquad\qquad (3)$$

According to these $\bar{x}$ and $\bar{y}$ mean components found, the mean resultant vector length is calculated as the following

$$\bar{R} = \sqrt{\bar{x}^2 + \bar{y}^2} \qquad\qquad (4)$$

and according to this equation, the mean direction is calculated by means of any of the following equations

$$\bar{\theta} = cos^{-1}\left(\frac{\bar{x}}{\bar{R}}\right), \qquad \bar{\theta} = sin^{-1}\left(\frac{\bar{y}}{\bar{R}}\right) \tag{5}$$

If it is $R=0$, the mean direction is undefined. In this case, it is stated that the data set is not concentrated in any direction on the unit circle and does not have any mean direction. If it is $R=n$, it is stated that the data set has a mean direction and that all observations are concentrated in the mean direction (6, 7).

Circular Variance: Since resultant vector length (R) is a measure of scattering which shows to what extent the observations are concentrated around the center, there is a close relationship between the variance, which is a measure of dispersion around the mean, and the resultant vector length in circular data (8).

A sample scattering related to random unit vectors is expressed by

$$D = n - \sum_{i=1}^{n} cos(\theta_i - \bar{\theta}) \tag{6}$$

Accordingly, if the dispersion around the mean direction is indicated by V, so it is

$$V = \sum_{i=1}^{n} cos(\theta_i - \bar{\theta}) \tag{7}$$

and if equation (7) is resolved, the sample variance for circular data is calculated as the following

$$V = \frac{1}{n} \cdot D$$
$$V = 1 - \bar{R} \tag{8}$$

In circular data, as in linear data, as the sample variance gets smaller, the distribution becomes homogenous. However, unlike linear variance, circular variance takes values between 0 and 1. If all observations are in the same direction, in other words, if there is no scattering, the mean resultant length will be close to 1, and accordingly, the variance will be minimum. If the observations are uniformly distributed on the perimeter of the circle, in other words, if scattering is maximum, then the mean resultant vector length will be 0, and accordingly, the variance will be 1 (9, 10).

Circular Standard Deviation: The sample standard deviation for directional data is calculated by the transformation of sample variance, similar to the standard deviation on the line (4). The sample standard deviation appropriate to the circular sample variance in the range (0,1) is defined as the following

$$v = \frac{180^o}{\pi}\sqrt{2(1 - \bar{R})} \tag{9}$$

Circular Standard Error: Circular standard error is a simple method which is used to determine the confidence interval when the sample size is ≥ 25.

The average of the data in the real part of the second trigonometric moment is calculated by the following equation

$$\bar{\alpha}_2 = \frac{1}{n}\sum_{i=1}^{n} cos2(\theta_i - \bar{\theta}) \tag{10}$$

and the standard error of mean direction is calculated (11) by the following equation

$$\sigma = \sqrt{\frac{n(1-\bar{\alpha}_2)}{2R^2}} \tag{11}$$

Concentration Parameter: The concentration parameter indicated by κ reflects whether the data set is homogeneously distributed on the circle or shows a concentration in the reference direction. It can be said that the data set is distributed uniformly on the circle when this value is 0 and that serious deviations from homogeneity occurred, in other words, data showed a concentration in the reference direction when it is greater than 2 (9). A suitable approach for the concentration parameter has been defined as the following

$$\hat{\kappa} = \begin{cases} 2\bar{R} + \bar{R}^3 + 5\bar{R}^5/6 & \bar{R} < 0.53 \\ -0.4 + 1.39\bar{R} + \frac{0.43}{(1-\bar{R})} & 0.53 \leq \bar{R} < 0.85 \\ 1/(\bar{R}^3 - 4\bar{R}^2 + 3\bar{R}) & \bar{R} \geq 0.85 \end{cases} \tag{12}$$

Circular Distributions: They are probability distributions in the 0-2π range on the unit circle. Although there are many distributions on the circle, studies on very few of them were carried out. The most important ones of these distributions are the uniform distribution which is the most basic distribution on the circle, and the von Mises distribution which is expressed as the circular normal distribution, which plays an important role in statistical inference (3).

Uniform Distribution: If the observations are uniformly distributed on the circle, this distribution is expressed as a circular uniform distribution. In circular uniform distribution, all directions have equal probability between 0° and 360°. Since all of the observations are equally distributed on the circle, the mean resultant length is equal to 0 and the variance of the distribution is equal to 1. Therefore, the mean direction is undefined in the circular uniform distribution. The most important feature of this distribution is that it is not affected by rotation and reflection (2).

In uniform distribution; probability density function:

$$f(\theta) = \frac{1}{2\pi}, \qquad 0 \le \theta < 2\pi \tag{13}$$

Mean direction	μ: undefined
Mean resultant length	$\bar{\rho}$: 0
Circular dispersion	δ: ∞
p. cosine moment	α_p: 0, $p \ge 1$
p. sine moment	β_p: 0, $p \ge 1$ (9).

Circular Normal Distribution (von Mises): If circular random variable θ has a normal distribution, the distribution is expressed as the von Mises distribution. The most important distribution on the circle in terms of statistical inference is the von Mises distribution (12).

Bessel function converted from the first type zero order is

$$I_0(\kappa) = \frac{1}{2\pi} \int_0^{2\pi} e^{\kappa \cdot cos(\theta - \mu)} \, d\theta \tag{14}$$

the probability density function of the von Mises distribution is

$$f(\theta; \mu, \kappa) = \frac{1}{2\pi I_0(\kappa)} e^{\kappa \cdot cos(\theta - \mu)} \tag{15}$$

and here, it is defined as $0 \le \theta, \ \mu \le 2\pi, \ \kappa \ge 0$.

The concentration parameter κ is a parameter that measures the concentration around the mean direction μ. Therefore, as the value κ increases, a high concentration also occurs around μ, which is the mean direction of the population (13). Furthermore, when von Mises distribution is $\kappa = 0$, it is converged to uniform distribution, for the small values of κ, it is converged to cardioid distribution, and when it is $\kappa > 2$, spiral is converged to normal distribution. The effect of $1/\kappa$ in circular normal distribution and the effect of σ^2 in normal distribution are almost the same.

In Von Mises distribution;

Mean direction	μ: $\bar{\theta}$
Mean resultant length	$\bar{\rho}$: $A_1(\kappa)$
Circular dispersion	δ: $[\kappa A_1(\kappa)]^{-1}$
p. cosine moment	α_p: $A_p(\kappa)$
p. sine moment	β_p: 0, $p \ge 1$'dir (9).

Hypothesis Tests for Mean Direction: An important question of circular statistics is how observations are distributed on the circle. It is stated that the distribution is not homogeneous if most of the data do not show a concentration around the mean direction, and that the distribution is homogeneous if the data has a uniform distribution on the perimeter of circle (14).

One-Sample Mean Direction Test: The confidence interval at significance level of $(1 - \alpha)$ can be used for one-sample mean direction test. It is necessary that the sample size should be at least 25 and the circular standard error should be determined for the hypothesis testing of mean direction and confidence interval. Whether the calculated mean direction is different from any mean direction given is tested.

The confidence interval for test statistics is calculated as the following

$$\mu_{gs} = \bar{\theta} \pm sin^{-1}\left(Z_{\frac{\alpha}{2}} \cdot \sigma\right)$$ (16)

If the mean direction is in the confidence interval given, hypothesis H_0 is accepted at a significance level of α (2).

Two-Sample Mean Direction Test: In this test, whether the mean directions of two circular distributions are different from each other is tested. The test statistics recommended by Watson and Williams is calculated (15) by the following equation

$$F_h = \left(1 + \frac{3}{8\kappa}\right)\left[\frac{(N-2)(R_1+R_2-R)}{N-(R_1+R_2)}\right]$$ (17)

Results

In this study, the representation methods of circular data, the calculation of descriptive statistics, mean direction, concentration parameters, hypothesis tests for compliance with uniform distribution and von Mises distribution, and hypothesis tests for the equality of mean directions, concentration parameters and distributions were introduced. Then, an application of these methods was performed with a data set including the times of birth of infants obtained from Van Yüzüncü Yıl University Faculty of Medicine Obstetrics and Gynecology Department.

The primary aim of the study was to introduce the circular data analysis which is not commonly used in practice and to show its applicability on a real data set. For this reason, in the discussion section of the study, biological interpretations of the results are not mentioned, and statistical interpretations are mainly emphasized.

Table 1. Circular descriptive statistics of the times of birth and hypothesis tests

n=179	Time (Hour) [360°]	
Actual Mean Direction ($\bar{\theta}$)	321.938	
Mean Resultant Length ($\bar{R}$)	0.0381	
Circular Variance (V)	0.9619	
Circular standard Deviation (v)	146.4416	
Circular Dispersion (δ)	305.6192	
Von Mises Concentration Parameter (κ)	0.0764	
Skewness (s)	0.0561	
Kurtosis (k)	-0.1048	
Mean Cos ($\bar{C}$)	0.0300	
Mean Sin ($\bar{S}$)	-0.0235	
Mean Cos ($\bar{C}_2$)	0.0281	
Mean Sin ($\bar{S}_2$)	0.1068	
Mean Direction $H_0=\theta$		
Score Test	Z= 0.1982	p= 0.6562
Likelihood Ratio Test	Z= 0.1981	p= 0.6563
Watson & Williams Test	F= 1.5013	p= 0.2221
Stephens Test	Z= 0.1982	p= 0.6562
Uniform Distribution Goodness-of-Fit Test	U^2=0.0792	p= 0.3840
Von Mises Distribution Goodness-of-Fit Test	U^2=42.8782	p= 0.0050

In Table 1, for the first trigonometric moments of the data, it was found that the mean cosine component ($\bar{C}$) was 0.03, the mean sine component ($\bar{S}$) was -0.0235, the mean resultant length ($\bar{R}$) was 0.0381 and the mean direction($\bar{\theta}$) was 321.938. For the second trigonometric moments, it was found that the mean cosine component ($\bar{C}_2$) was 0.0281, and the mean sine component ($\bar{S}_2$) was 0.1068. These values are used in the calculation of circular descriptive statistics and tests. These statistics are needed to perform various tests on Von Mises distribution parameters.

The mean direction ($\bar{\theta}$), one of descriptive statistics of the times of birth of infants, is the expression of the average value of the data distributed on the circle in degrees, and this value was calculated as 321.938°. Accordingly, the mean time of birth is approximately 21:28. The mean resultant length($\bar{R}$) is the mean length of the resultant of all observations and is a measure determining the concentration. This value is between the range of (0,1). A value close to 1 indicates a high concentration, and a value close to 0 indicates that there is no concentration and that the data is uniformly distributed around the circle. Since it is $\bar{R} = 0.0381$, it is seen that the data is uniformly distributed on the circle, in other words, the times of birth are not concentrated at any time of the day. V is the circular variance, and this value is descriptive of the spread in the data set. Circular variance takes value between the range of 0 and 1. In the sample, the fact that circular variance gets close to maximum with the value $V = 0.9619$, indicates a high spread of observations on the circle. Circular standard deviation (v) refers to deviations from the mean direction. It was calculated to be $v = 146.4416^o$. Another measure of dispersion (δ) based on the first and second central trigonometric moments is the measure of circular scattering, and this value was calculated as 305.6192. The fact that the concentration parameter(κ), which shows the concentration of circular data on the circle, was calculated as 0.0764 indicates that the data was uniformly distributed on the circle.

With respect to skewness and kurtosis parameters, the fact that the circular skewness value is found to be close to zero indicates a symmetric single-mode data set around the mean direction. The fact that the kurtosis value is less than 0 indicates that the distribution is more kurtic, flat and that the data is heterogeneous.

The hypothesis under the Von Mises assumption is established as

H$_0$: The mean direction under the circular normal distribution is equal to 0°

H$_1$: The mean direction under the circular normal distribution is different from 0°.

Under this assumption, score, Likelihood ratio, Watson & Williams and Stephens test statistics are given. According to the four tests given, hypothesis H$_0$ is decided to be accepted, and it can be said that the mean direction of the times of birth is not different from the direction 0° at a significance level of $\alpha = 0.05$.

The Watson test statistic was used to test whether the times of birth were uniformly distributed over 24 hours a day or complied with the normal distribution. For uniform distribution, hypothesis H$_0$ is accepted and it can be said that the times of birth are uniformly distributed on the circle at a significance level of $\alpha = 0.05$. For Von Mises distribution, (p<0.05) hypothesis H$_0$ is refused. Thus, it can be said that the sample is not compatible with the circular normal distribution (von Mises distribution) at a significance level of $\alpha = 0.05$.
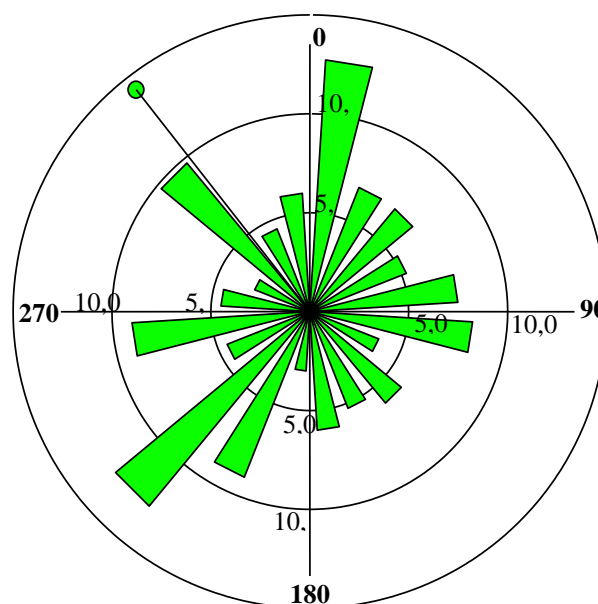

Figure 2: Rose diagram of the times of birth

The rose diagram for circular data on the times of birth is presented in Figure 2. When the figure is examined, it can be observed that the data is uniformly distributed on the circle, and that the mean direction is between 270° and 360°. When the range observed in the graphic is further reduced to a range of 305° to 335°, it can be said that the mean direction of the times of birth is approximately between 20:20 and 22:20.

Table 2. Circular descriptive statistics of the times of birth according to gender and hypothesis tests

n=179	Time (Hour) [360°]			
	Male (n=104)		**Girl (n=75)**	
Actual Mean Direction	285.0255		328.9989	
Mean Resultant Length	0.0116		0.0788	
Circular Variance	0.9884		0.9212	
Circular standard Deviation	171.0192		129.1738	
Circular Dispersion	3035.6413		67.451	
Von Mises Concentration Parameter	0.0233		0.1588	
Skewness	-0.1811		0.1742	
Kurtosis	-0.0250		0.0638	
Mean Cos	0.0030		0.0675	
Mean Sin	-0.0112		-0.0406	
Mean Cos	-0.0680		0.1614	
Mean Sin	0.1663		0.0245	
Mean Direction $H_0 = \theta$				
Equal Distributions	Test Statistics= 0.4842		p= 0.7850	
Equal Directions	Test Statistics= 1.7478		p= 0.1879	
Equal Concentration Parameters	Test Statistics= 0.3756		p= 0.5400	
Score Test	Z= 0.0262	p= 0.8714	Z= 0.2476	p= 0.6188
Likelihood Ratio Test	Z= 0.0262	p= 0.8714	Z= 0.2474	p= 0.6189
Watson & Williams Test	F= 0.8973	p= 0.3457	F= 0.9036	p= 0.3449
Stephens Test	Z= 0.0262	p= 0.8714	Z= 0.2476	p= 0.6188
Uniform Distribution Goodness-of-Fit Test	U^2=0.067	p= 0.4636	U^2=0.0765	p= 0.4021
Von Mises Distribution Goodness-of-Fit Test	U^2=24.609	p= 0.0050	U^2=17.819	p= 0.0050

In Table 2, the data set of the times of birth was divided into two groups according to the gender of infants, and two samples were obtained by gender. The sample size was 104 baby boys and 75 for baby girls. The descriptive statistics and mean direction tests of these two samples, von Mises and normal distribution compliance tests, and the most common tests used to compare two groups were given to determine whether the times of birth varied by gender.

The value $\bar{\theta}$ calculated for the first sample indicates that the mean time of birth for baby boys is approximately 19:00. Since it is $\bar{R}$ =0.0116, it can be said that the data was uniformly distributed on the circle, in other words, the times of birth of baby boys were distributed over 24 hours of a day. The fact that the circular variance is close to maximum and the fact that the concentration parameter takes a small value like 0.0233 indicate that all observations were uniformly distributed on the circle. It was found that the circular standard deviation was 171.0192° and that the measure of circular dispersion, which is another measure of spread, was 3035.6413.

Although the skewness value of the first sample shows that the distribution is slightly skewed to the left, it can be said that this distribution is symmetric single-mode around the mean direction since it is close to zero. Similarly, although the kurtosis value also shows that the distribution is slightly flattened, it can be said that the circular kurtosis of distribution is the same as normal distribution since this value is close to zero.

The score, Likelihood ratio, Watson & Williams and Stephens test statistics were given to test whether the mean direction of the times of birth of baby boys was different from the H_0 mean direction (0°). According to these four tests given, hypothesis H_0 is decided to be accepted. Thus, it can be said that the mean direction of the times of birth of 104 baby boys (285.0255°) is not different from the direction 0° at a significance level of $\alpha = 0.05$.

The Watson test was given to test whether the times of birth of baby boys were uniformly distributed or were compatible with the von Mises distribution. For uniform distribution, hypothesis H_0 is accepted. Accordingly, it can be said that the times of birth of baby boys were uniformly distributed around the circle at a significance level of $\alpha = 0.05$. For Von Mises distribution (p<0.05), hypothesis H_0 is refused. Therefore, it can be said that the sample is not compatible with the circular normal distribution at a significance level of $\alpha = 0.05$.

The value $\bar{\theta}$ calculated for the second sample indicates that the mean time of birth of baby girls is approximately 21:56. Since it is $\bar{R} = 0.0788$, it can be said that the data was uniformly distributed around the circle, in other words, the times of birth of baby girls were distributed over 24 hours of a day. The fact that the circular variance is 0.9212 and the fact that the concentration parameter was found to be 0.1588 indicate that the data was uniformly distributed around the circle. Furthermore, it was found that the circular standard deviation was 129.1738° and that the measure of circular dispersion was 67.451.

The skewness value given for girls indicates that the distribution is slightly skewed to the right. However, it can be said that the circular distribution is symmetric single-mode around the mean direction since this value is close to zero. It can be said that the kurtosis value is distributed slightly steeper than normal, but, the circular kurtosis of distribution is the same as normal distribution since this value is very close to zero.

The score, Likelihood ratio, Watson & Williams and Stephens test were given to test whether the mean direction of the times of birth of baby girls was different from the H_0 mean direction (0°). According to these four tests given, hypothesis H_0 is accepted. Thus, it can be said that the mean direction of the times of birth of baby girls (328.9989°) is not different from the direction 0° at a significance level of $\alpha = 0.05$.

The Watson test was used to test whether the times of birth of baby girls were uniformly distributed or were compatible with the von Mises distribution. For uniform distribution, hypothesis H_0 is accepted. Accordingly, it can be said that the times of birth of baby girls were uniformly distributed around the circle at a significance level of $\alpha = 0.05$. For Von Mises distribution (p<0.05), hypothesis H_0 is refused, and it can be said that the sample is not compatible with the circular normal distribution at a significance level of $\alpha = 0.05$.

The Score test was used for the equality of distributions, the Watson-Williams F-test was used for the equality of mean directions, and the Concentration homogeneity test was used for the equality of concentration parameters. According to the Score test, hypothesis H_0 was accepted, and thus, it can be said that both sample distributions are equal to each other at a significance level of $\alpha = 0.05$. According to the Watson-Williams F-test, hypothesis H_0 was accepted. Thus, it can be said that there is no difference between the mean directions of both samples at a significance level of $\alpha = 0.05$. According to the Concentration homogeneity test, hypothesis H_0 was accepted, and it can be said that sample concentration parameters are equal to each other at a significance level of $\alpha = 0.05$.
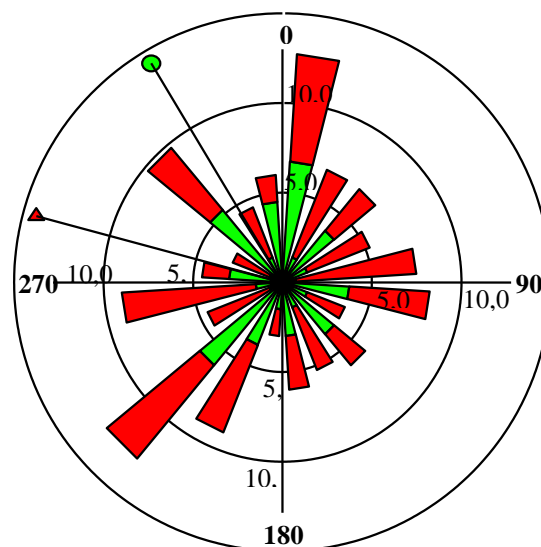

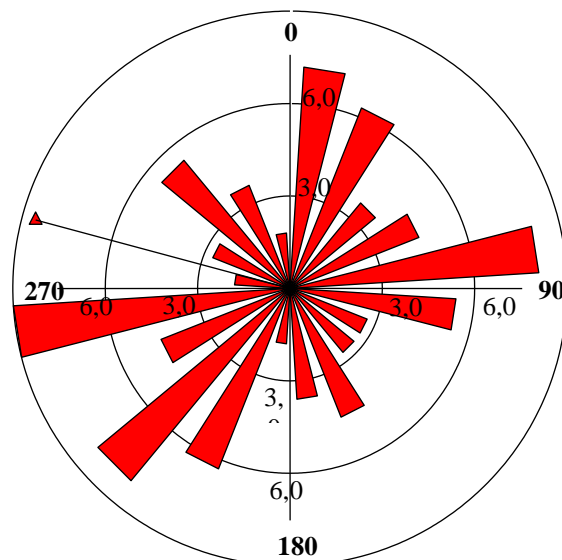**Figure 3:** Rose diagram for the times of birth according to baby's gender

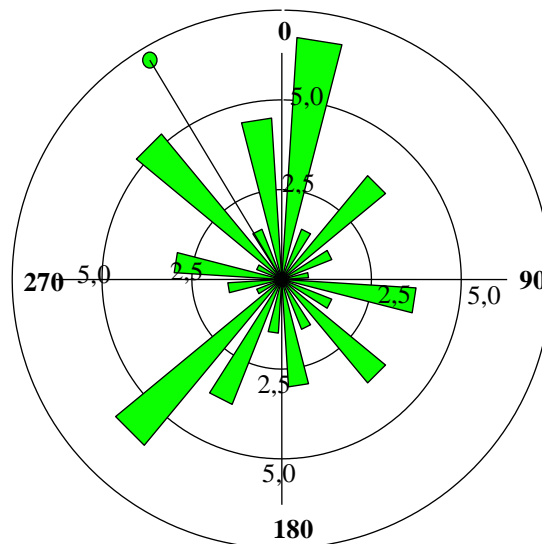**Figure 4:** Rose diagram for the times of birth of baby boys

Figure 5: Rose diagram for the times of birth of baby girls

Baby boys are represented in red and baby girls are represented in green, and the rose diagram for the times of birth according to baby's gender is presented in Figure 3. The rose diagram for the times of birth of only baby boys is presented in Figure 4 while the rose diagram for the times of birth of baby girls is presented in Figure 5. When each graph given is examined separately, it can be said that the data was uniformly distributed on the perimeter of circle, in other words, there is no significant difference between the times of birth. The mean directions for the times of birth of baby boys and girls were observed between 270° and 360°. This range can be further narrowed and it can be said that there is no significant difference between the mean direction of the times of birth of baby boys and the mean direction of the times of birth of baby girls.

Discussion

Since the aim of the present study was to give information about circular data, descriptive statistics and hypothesis tests and to explain their functionality with an application, numerical values were not included in this section.

Since the validity of statistical data analysis methods to be used in a scientific research is directly related to the data structure and scale type, it is clear that the same methods cannot be applied to the data obtained from each scientific

research, and therefore, it is necessary to select an analysis method which is appropriate to the scale type and the relevant data structure.

The fact that the topographic structures of the circle and the line are different from each other makes the data structures and scale types defined on them different from each other. Therefore, since the application of linear statistical methods to the data which is represented by an angle or is in the time

cycle, in other words, on a circle or sphere surface will mostly produce false or misleading results, it is clear that it will be more accurate to use circular statistical methods in such periodic data.

It has been shown that there are significant differences between circular statistical methods and standard linear statistical methods, and that if a circular data set is attempted to be explained by standard linear statistical methods, the results may be inaccurate. Furthermore, the difficulties in the interpretation of descriptive statistics found by standard linear statistical methods were also mentioned. It is also necessary to emphasize that the results obtained will be meaningless in some cases.

It was pointed out that these important differences observed for descriptive statistics were also valid for test statistics and that the tests performed did not give proper results in some cases. Nevertheless, it was mentioned that the figures did not express a process since the point of time measured did not have an initial value no matter in which unit it is (such as seconds, minutes, hours, days, weeks, months, years).

Conclusion

it was shown that circular data cannot be analyzed by the analysis methods developed for standard linear data due to several reasons and that the results obtained in case of using standard methods could be inaccurate or could be meaningless in some cases, and therefore, it was emphasized that appropriate statistical methods should be used.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: **YD, ÖCB:** Research concept and design; data collecting, **YD:** Preparation of article, and Revisions. All authors approved the final version of the manuscript

Ethical issues: All Authors declare, originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities.

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A Case of Primary Malignant Melanoma of the Larynx

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Abstract

Objective: Malignant melanoma of the larynx is an uncommon tumor type that can be seen as a primary tumor or as a metastasis from a cutaneous primary lesion. Morphological appearances of this tumor are readily confused with the other types of laryngeal cancers. A new case of primary laryngeal malignant melanoma has been presented in this case report .

Case: The patient was a 54-year-old man without any clinical evidence of other cutaneous malignant melanocytic lesions. Microscopically, polygonal-epithelioid tumor cells containing cytoplasmic and nuclear melanin were located under the mucosal squamous epithelial cells. Atypical mitotic figures were also present. Ulceration and disruption of the epithelium weren't observed. Immunohistochemical studies were positive for S100, HMB-45, Melan-A and vimentin while it was negative for cytokeratin. Based on findings this diagnosis was established.

Keywords: Mucosal malignant melanoma, laryngeal primary melanoma, malignant melanoma.

Introduction

Malignant melanomas are malignant tumors, most of them with cutaneous origin. Compared with cutaneous melanomas of the head and neck, primary melanoma of the upper airways and digestive tract has poor prognosis. In the medical literature only a few cases of mucosal derived malignant melanoma have been reported. Mucosal malignant melanoma of the upper aero digestive tract represents 0.5% to 3% of all malignant melanomas while laryngeal melanomas represent up to 3.6-7.4% (1). Therefore, knowledge about clinicopathologic features, treatment protocols and prognosis of mucosal malignant melanomas are not clearly established.

In this report, we define a case of primary malignant melanoma of the larynx.

Case

A 54-year-old man was admitted to our hospital with external center biopsy result. At the time of admission, he had suffered recurrent episodes of hoarseness in the past 6 months. He had no other complaints or symptoms. The patient admitted smoking average of 20 cigarettes a day for more than 30 years. He was also an occasional drinker. Computed tomography scan of the larynx demonstrated the presence of 7 mm diameter nodular lesion in anterior commissure. PET scan of the patient revealed that only larynx show up as bright spots.

After his first diagnosis in a center, he made an application to our laboratory with 4 formalin fixed paraffin-embedded blocks and 4 H&E slide preparations. On the routine HE staining, histopathologic examination larynx mucosal epithelium was intact. Ulceration and disruption of the epithelium weren't observed (Figure 1 and 2). Tumor had a predominantly solid growth pattern infiltrating submucosal compartments and located in the subepithelium. The tumor cells containing cytoplasmic and nuclear melanin, demonstrate pleomorphic eosinophilic cytoplasm with big and round vesicular nuclei with one or many nucleoli (Figure 3). Some areas of the tumor were consisted of spindle-shaped cells, while the others showed polygonal to round epithelioid cells. There was marked cellular pleomorphism, increased mitotic activity. The depth of tumor invasion was 3 mm. Pagetoid spread wasn't observed therefore the tumor was thought to be a nodular melanoma according to these findings. Lymphovascular invasion was not observed.

Immunohistochemical studies were positive for S100, HMB-45, Melan-A and vimentin while cytokeratin and iron stains were negative (Figures 4). Extensive physical examination by a clinician failed to reveal a primary cutaneous lesion.Finally, the patient was diagnosed with primary malignant melanoma of the larynx. Despite all additional treatments, the patient died 30 months later.
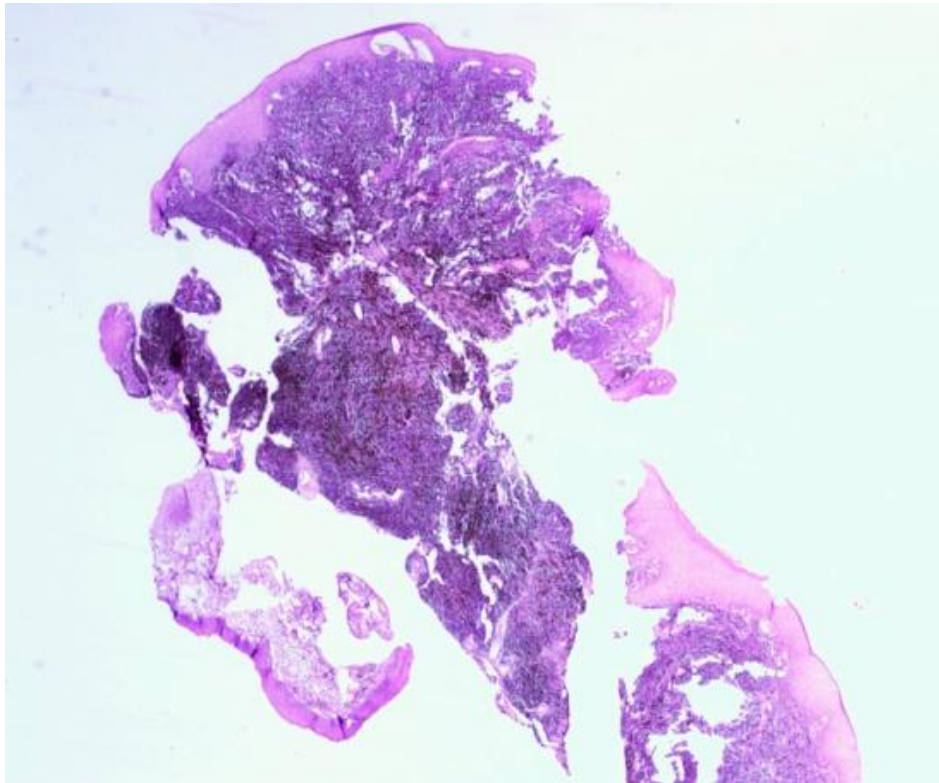
Figure 1: Malignant melanocytes under the epithelium (HE staining 4x)
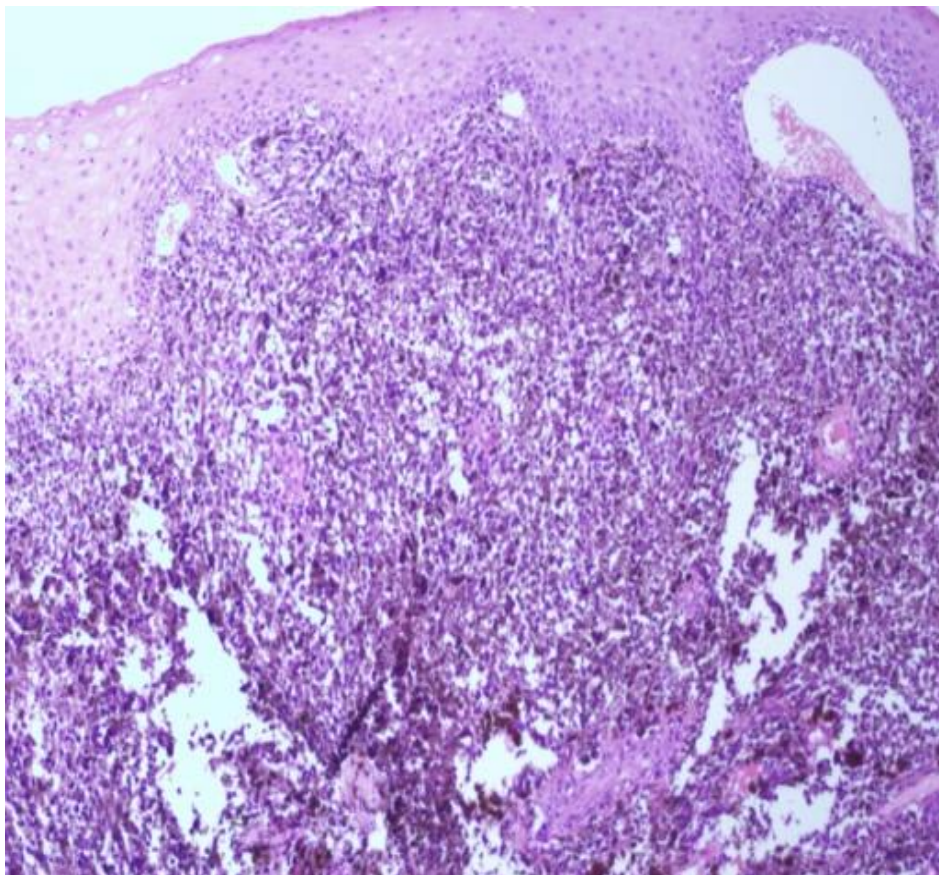

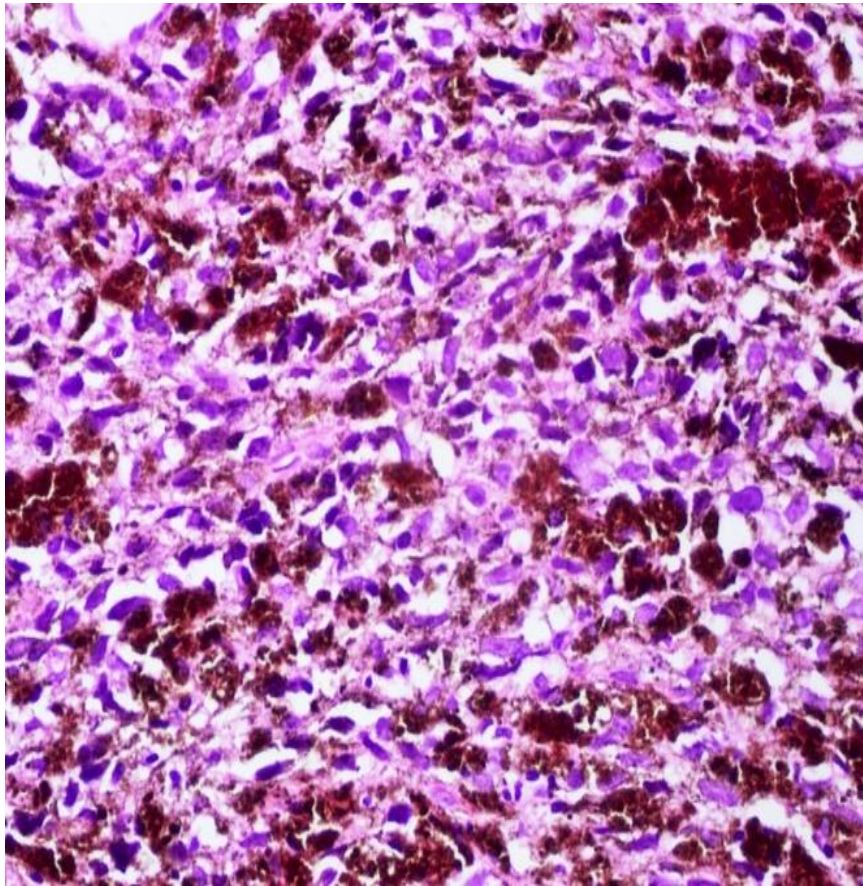**Figure 2:** Intact mucosal epithelium, no ulceration (HE staining 10x)

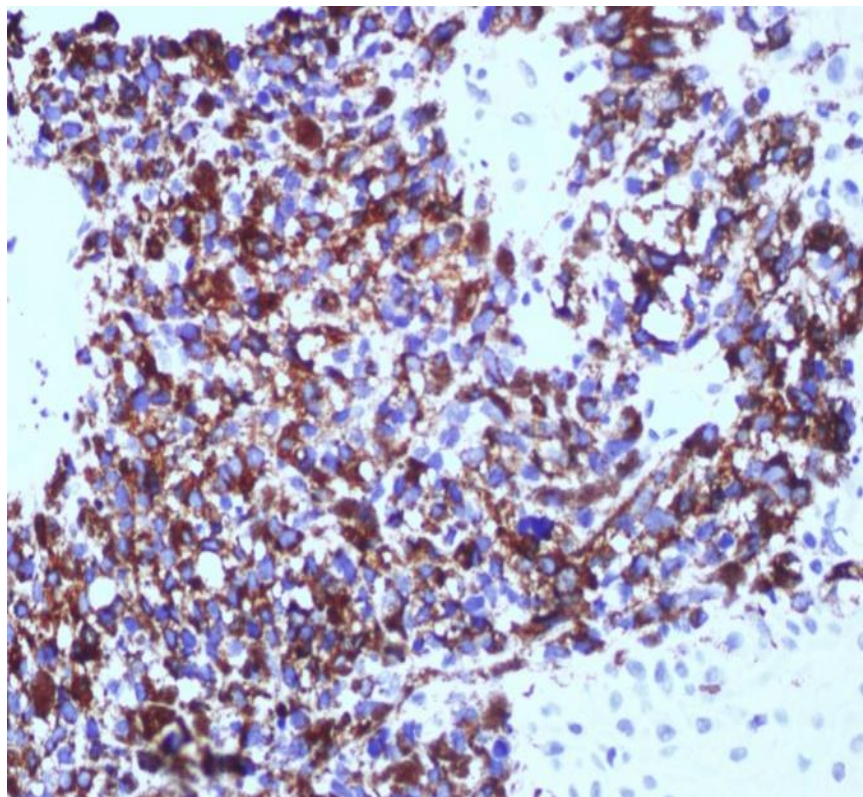**Figure 3:** Malignant spindle-shaped cells mixed with pigmented cells (HE staining 40x)

Figure 4: Immunoreaction for HMB-45 showing strong positivity staining of the tumor cell (40x)

Discussion

Melanocytes are originate from a neuroectodermal-derived structure called neuronal crest and localize in the cutaneous and non-cutaneous tissue such as leptomeninges, uvea, gastrointestinal, respiratory and genitourinary tracts (1,2). Mucosal melanoma of the larynx is extremely rare tumor with 60 cases reported in the medical literature, but it is more common than metastatic types (2). It is difficult to distinguish primary mucosal melanoma from the metastasis of an unknown cutaneous tumor. Histopathologically, primary melanoma tumors are related to overlying mucosa with junctional activity, but metastatic melanoma do not have any junctional changes with adjacent intact mucosa (6). In this case, mucosa was intact and there was junctional activity between tumor and overlying mucosa. For differentiation of primary lesion from metastasis, revealing a primary cutaneous lesion is also important.

Mucosal melanomas, which are biologically distinct from lesions of cutaneous origin, are caused by unknown factors. Etiological factors, including melanocytosis are still remained speculative (4).

Mucosal melanomas are more aggressive than cutaneous tumors with five-year survival of less than 10% (5). At the time of diagnosis, having distant metastasis is the cause of poor prognosis (6).

Accurate diagnosis of primary mucosal melanoma of the larynx can be challenging for multiple reasons. Patients may present with non-specific symptoms of hoarseness or hemoptysis. The differential diagnosis of laryngeal masses includes squamous cell carcinoma, neuroendocrine carcinoma, paraganglioma, lymphoma and granulomas. Especially, poorly differentiated squamous cell carcinoma (SCC) of the larynx must be differentiated from melanoma. Melanomas are distinguished from squamous cell carcinoma by its expression of S100, HMB45, melanA and other melanocytic markers (4-7).

The treatment for mucosal melanomas of the head and neck, require complete surgical resection with negative margins (7). Recent studies have shown that postoperative radiation therapy to the affected area may improve the local control, but it is not clear if this translates to an improvement in prognosis (2). Biochemical, targeted therapies and therapeutic drugs directed at genetic mutations are now also under investigation and available for patients (7).

Conclusion

Primary mucosal malignant melanoma is a rare and highly aggressive tumor. The diagnosis of melanoma can be very challenging for histopathological examination therefore immunohistochemical stains are essential. At the time of diagnosis, any other primary cutaneous lesions need to be excluded.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: **ÖK, DSK, GD, GD:** Research concept and design; data collecting, patient examination **ÖK:** Preparation of article, and Revisions. All authors approved the final version of the manuscript

Ethical issues: All Authors declare, originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities.

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