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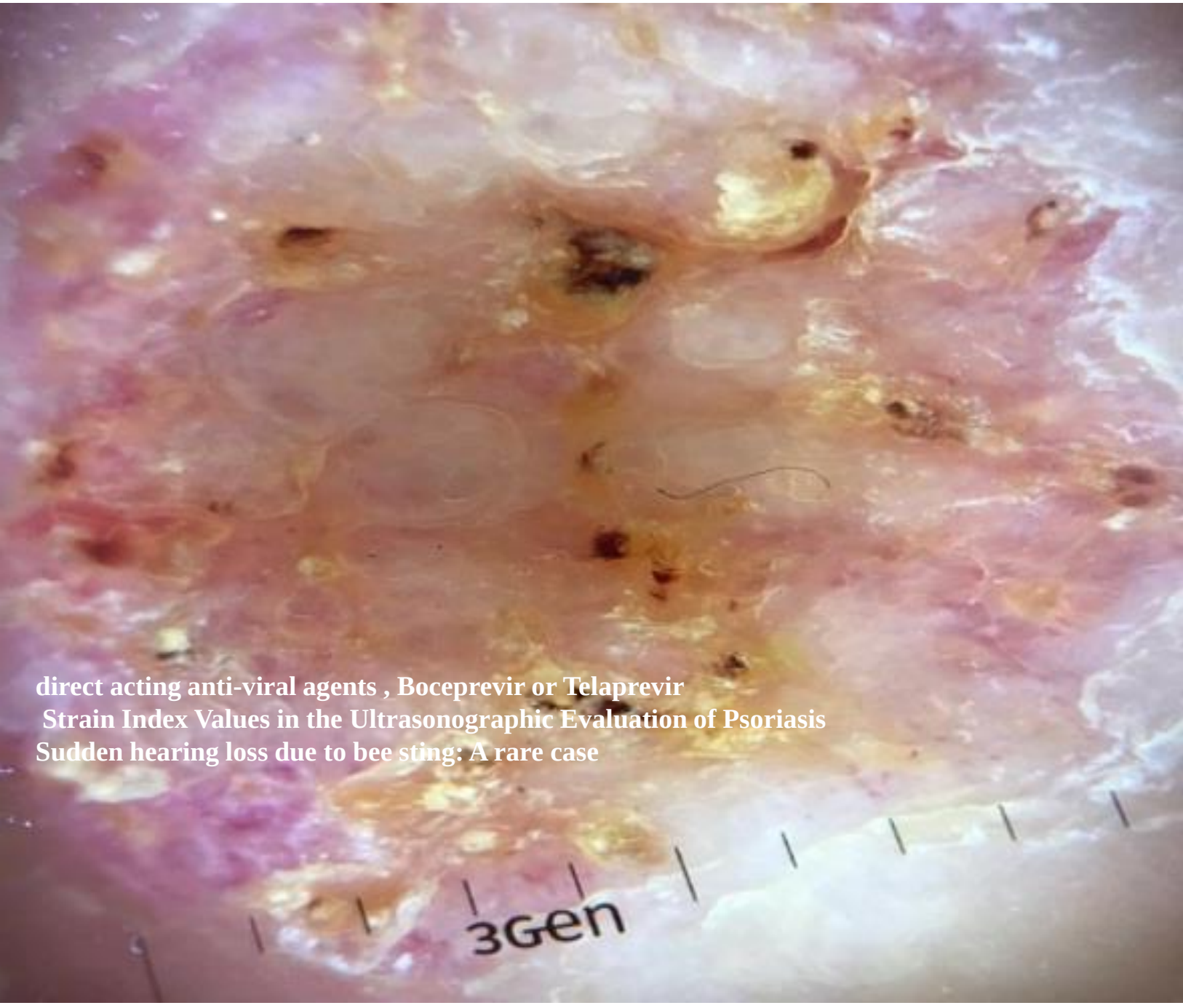

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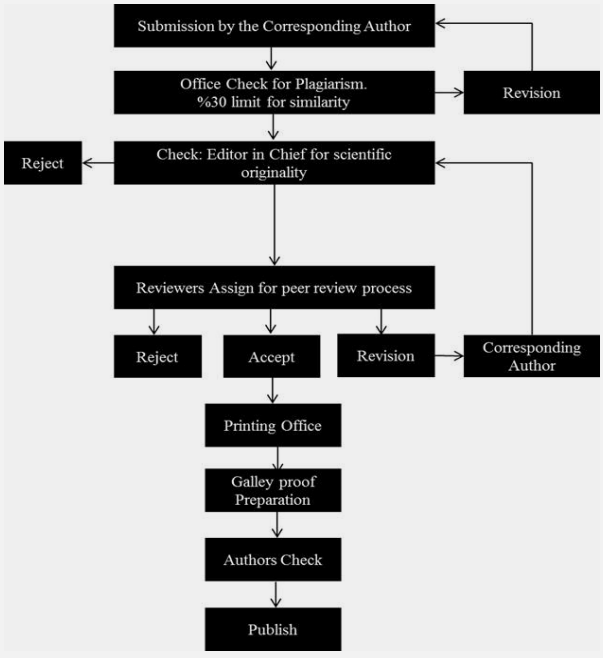

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The Effects of EGFR Exon 19 747–750 Deletion on the Risk of Developing Lung Cancer

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Abstract

Objective: Although smoking is the most significant factor in the etiology of lung cancer, other environmental pollutants and genetic predisposition also play major roles in its development. Histopathologically, lung cancers are divided into two major types, as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The latter accounts for almost 85% of all lung cancers with a very aggressive course and being associated with a high rate of mortality. Among the genetic mutations with prognostic value in NSCLC, the epidermal growth factor receptor (EGFR) mutation is most frequently found in 50 to 80% of cases. The EGFR is a transmembrane glycoprotein with tyrosine kinase activity which is associated with both normal cell growth and malignant transformations.

Material and Methods: In the present study, we aimed to evaluate the effects of exon 19 747–750 deletion in the EGFR gene on the risk of developing lung cancer and to examine its potential relationship with the different histopathological types of lung cancer. The study sample comprised a total of 178 patients diagnosed with lung cancer at Mersin University, Medical Faculty, Oncology Clinics, and 192 age- and sex-matched healthy individuals as the control group. Deoxyribonucleic acid (DNA) isolation was performed using the standard salt-water precipitation method, while the mutation screening and genotyping analyses were carried out with a polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses.

Results: The frequency of mutant EGFR exon-19 deletion in the control group was 15.1%, increasing to 36.9% in the lung cancer group, and increasing the risk of developing lung cancer by 2.64 times (p: 0.014). This increase did not significantly differ between the histopathological types of lung cancer (p: 0.76).

Conclusion: Considering the distribution of lung cancer patients in different age groups, it is obvious that advanced age is a risk factor for the development of EGFR mutation and lung cancers (p<0.001).

Key words: Lung cancer, EGFR gene, Exon 19 deletion, Older age.

Introduction

Lung cancer is the most frequent among all types of cancer and is associated with the highest rate of mortality (1). Lung cancers can be divided into two types based on cell morphology, as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Globally, more than 1.6 million individuals die from lung cancer annually (2), with almost 85% of the total attributable to NSCLC, and the remainder to SCLC. Smoking is the most important factor in the etiology of lung cancer, although other environmental pollutants and genetic predisposition also play major roles in its development (3).

Of note, NSCLC follows a very aggressive course and is associated with a high rate of mortality, being the main type of lung cancer, but incorporating several other histopathological types of lung cancer(4). In adenocarcinomas, the rates of epidermal growth factor receptor (EGFR) gene mutations, Kristen-rat sarcoma oncogene (K-RAS) mutations and mesenchymal–epithelial transition (MET) gene mutations have been reported as 20 to 30%, 30%, and 5%, respectively (5).

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The EGFR is one of the most important proteins playing a role in the cell differentiation and proliferation with an activity that involves its binding to the surface receptors of the EGFR. It is a transmembrane glycoprotein with tyrosine kinase activity which is associated with both normal cell growth and malignant transformations (6). Previous studies have shown an overexpression of EGFR in the presence of lung, head and neck, colon, pancreas, breast, ovary, bladder and kidney cancers, and in gliomas (7,8). Using the immunohistochemical methods, the EGFR overexpression has been found in 39%of adenocarcinomas, 58% of squamous-cell carcinomas, and 38%of large-cell carcinomas (9). This is of prognostic importance, in that it may contribute to the survival, recurrence and selection of appropriate treatment protocols. In the present study, we aimed to evaluate the relationship between the 747–750 deletion mutations in exon 19, the risk of lung cancer development and to examine the co-existence of the relevant mutations with specific histopathological types of lung cancer, as well as its relationship with other risk factors for lung cancer, including smoking, sex, and old age.

Material and Methods

Study population

The study sample comprised a total of 178 patients diagnosed with NSCLC at Mersin University, Medical Faculty, Oncology Clinics and a control group of 192 age- and sex-matched healthy volunteers between 2013 and 2015. All participants were informed about the objective and design of the study and a written informed consent was obtained from each participant. Data including age, occupation, smoking status and family histories of lung cancer were recorded on an information collection form which was designed specifically for the study. The interview response-rates among the eligible patient and control participants were 97.2% and 98.3%, respectively. The study protocol was approved by the Local Ethics Committee of Mersin University, Medical Faculty of Medicine. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Genotype analyses: Peripheral blood samples of 7 to 8 mL were obtained from each participant and placed in 15 mL centrifugation tubes containing 50 mmol/l disodium-ethylenediaminetetraacetic acid (EDTA). Deoxyribonucleic acid (DNA) isolation was carried out through the standard salt precipitation method (10), while the mutation screening and genotyping analyses were performed through polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses. After the obtained PCR/RFLP products were inspected through electrophoresis, the collected data were statistically analyzed.

EGFR Exon 19(747–750) Deletion Studies: EGFR gene mutation was identified by PCR-restriction fragment length polymorphism in 178 lung cancer patients. The sequences of primers for PCR amplification were as follows:

Forward:

5’- ATCCCAGAAGGTGAGAAAGATAAAATTC -3’

Reverse:

5’- CCTGAGGTTCAGAGCCATGGA -3’

The 20 µl PCR system of the first run contained 20 ng DNA, 1.5 mmol/l MgCl2, 1×PCR buffer, 200 nmol deoxynucleotide triphosphates, 200 nmol/l PCR primers and 0.2 U TaqDNA polymerase (Qiagen, Holland). This grade was to present restriction sites of restriction enzyme MseI by primer EGFR. PCR appliance (2720 Thermal cycler, Applied Biosystems) was designed for amplification. The PCR cycling parameters were: one cycle of 95°C for 15 minutes, 40 cycles of 95°C for 20 seconds, 60°C for 30 seconds, and 72°C for 1 minute, followed by one cycle of 72°C for 3 minutes. The product of PCR was 138 bp. PCR product (2 µl) containing 200 ng DNA was digested by MseI (MBI Fermentas, USA) at 37°C for 2 h. The digested 10 µl products were examined on a 3% agarose gel electrophoresis and staining with ethidium bromide. The results were analyzed by a UV imaging system. The digested fragments of wild-type DNA included 92 bp and 46 bp, and the digested fragments of mutant DNA included 120-129 bp. The principle of the assay is shown in the MseI which was used to digest the TTAA sequence (from first letter of codon 747 to first letter of codon 748) in wild-type genes, which are frequently absent in exon 19 deletion mutants (codons 747–750; Leu Arg-Glu sequence), resulting in the enrichment of deletion-type genes (12,19).

Statistical Analysis: The relationship between positivity in genes and disease were calculated by chi-square test. Distribution of lung cancer in age groups was determined by one sample chi square test. Categorical variables were expressed as frequencies and percentage. Statistical analysis was performed using the STATISTICA (13.3.1) $p<0,05$ was considered statistically significant.

Results

The demographic characteristics and frequencies of alleles/genotypes of EGFR exon 19 deletion (residues 747–750) of the patients with lung cancer and the healthy control sare presented in Table 1.

The mean age in the patient group was slightly higher than that of the control group, being 60.06(±9.75) years vs.58.04(±8.64) years, respectively (p:0.414). Of all the 178 patients with lung cancer, 155(86.6%) were men and 23(13.4%) were women. Lung cancer was more frequent among men (0.001). Smokers accounted for 64.50% and 80.3%of the control and patient groups, respectively ($p<0.001$). When EGFR 19 exon 747–750 deletion genotype rates were analyzed, the rate of mutant genotype was found to be 15.1%in the control group, increasing to 36.9%in the patient group, and a 2.64-times greater risk of developing lung cancer (p:0.014).The distribution of lung cancer patients based on the histopathological types and EGFR exon 19 747–750 deletion rates were as follows: Adenocarcinoma 53.37–52.08%, squamous cell carcinoma 30.89–35.41%, large cell carcinoma 3.93–4.16%, and small cell carcinoma 11.79–8.33%(p:0.76) (Table 2). Agarose gel electrophoresis of PCR products that wild and mutant genotype for EGFR exon 19 was demonstrated in Figure 1.

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Table 1. Distribution of study participants according to cases-control status, demographic characteristics and EGFR exon 19 deletion genotypes

Variables		Cases (n=178) (Mean ± SD)	Controls (n=192) (Mean ± SD)	p-value
Age (Mean ± SD)		60.06±9.75	58.04±8.64	0.414
sex	Male	155 (86.60)	162 (84.40)	0.001
	Female	23 (13.40)	30 (15.60)	
Smoking status	Never smokers	33 (19.70)	68 (35.50)	0.001
	Current smokers	145 (80.30)	124 (64.50)	
Genotype frequencies		112 (63.1)	163 (84.9)	0.014
		66 (36.9)	29 (15.1)	

Table 2. Distribution of EGFR exon 19 deletion (residues 747–750) genotypes based on histopathological types of lung cancer

	Adenocarcinoma		Squamous cell carcinoma		Large cell carcinoma		Small cell carcinoma	
	n	%	n	%	n	%	n	%
Total (n=178)	95	53.37	55	30.89	7	3.93	21	11.79
Wild type (n=130)	70	53.84	38	29.23	5	3.84	17	13.07
Mutant (n=48)	25	52.08	17	35.41	2	4.16	4	8.33

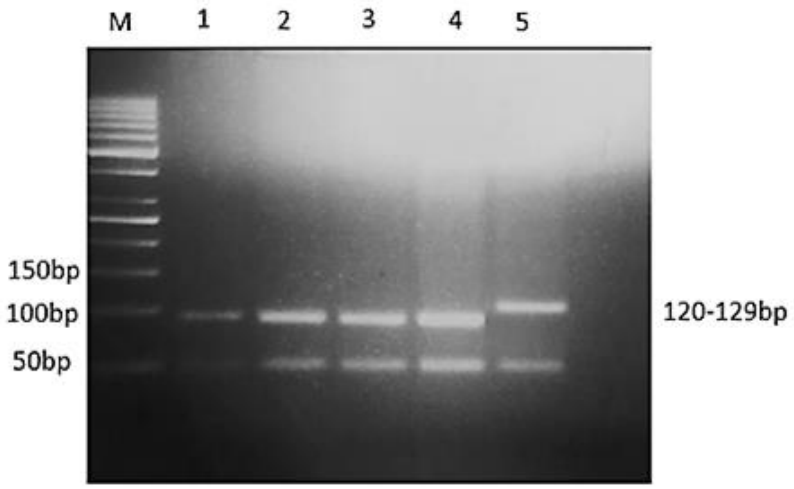

Figure 1: Agarose gel electrophoresis of PCR products for sequencing EGFR exons 19 gene by RFLP method. M: DNA marker; 1-4: patients wild type DNA sample; 5: patient mutant type DNA sample.

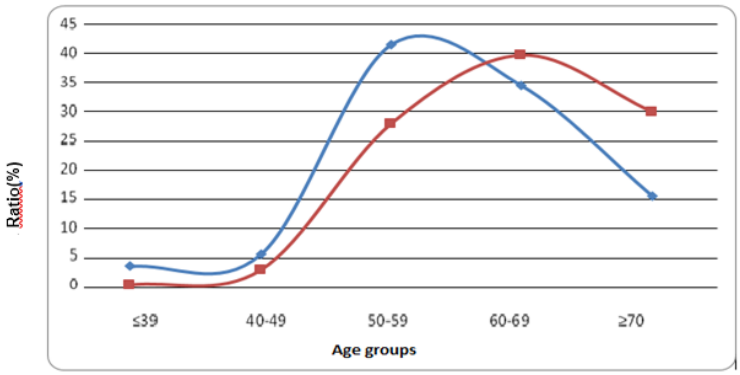

Figure 2. Blue line: Distribution of ratio of Lung cancer patients with respect to age. Red line: Distribution of ratio of Lung cancer patients with respect to older age in the Mersin sample.

The mean age of the 178 patients in lung cancer group was 60.06(±9.75) years with a distribution of patients according to the age groups being 3.3% in <39 years age group, 5.0% in 40–49 years age group, 39.2% in 50–59 years age group, 33.7% in 60–69 years age group, and 18.7% in >70 years age group. To identify whether old age represented a risk factor, the distribution of the respective age groups in the total Mersin population was initially estimated. As the population was not distributed evenly between the all age groups, the ratios of all lung cancer patients in all age groups were calculated. These ratios were as follows: 0.4% in the ≤39 years age group, 2.93% in 40-49 years age group, 27.9% in 50-59 years age group, 38.6% in 60-69 years age group and 29.9% in ≥70 years age group. When the ratio of the overall population and lung cancer patients in the relevant age groups was compared, old age was found to be a risk factor for the development of lung cancer (p:0.001).

To evaluate the increased risk of lung cancer in the older age groups, case numbers and ratios of the relevant age groups were estimated within the Mersin population (Fig. 2). We found no relationship between being in an older age group and the risk of lung cancer (Fig.2, blue line), although the ratio of lung cancer patients increased with older age in the Mersin sample (Fig.2, red line). Consistent with the literature, lung cancer was directly associated with smoking.

Discussion

In this population-based case-control study, we investigated whether the exon 19 deletion (residues 747–750) in the EGFR gene was related to the risk of lung cancer in a Turkish population, and whether there was any relationship between the EGFR exon 19 deletion genotypes and the histopathology types of lung cancers. We further investigated whether demographic risk factors influenced lung cancer development in our study sample. Based on our study results, the EGFR exon-19 747–750 deletion mutant genotype and older age were the main risk factors for the development of lung cancer.

In a previous study by Dearden et al. (11), the frequency of EGFR mutations in the Western populations was found to be 19.2% in patients with adenocarcinoma and 3.3% in patients with squamous cell carcinomas. These rates are higher in the Asian population, reported to be 47.9% in patients with adenocarcinomas and 4.6% in patients with squamous cell carcinomas. Asano et al. (12) identified exon 19 mutations in the EGFR gene in 52% of 108 patients with lung cancer, including mutations identified by direct sequencing in 16 (15%) of the 108, from a fine-needle lung biopsy sample in four (22%) of 18, and from a pleura fluid sample in three (15%) of 20 patients. In another population-based study performed by Shiau et al. (13) in Ontario State, Canada, EGFR exon 19 deletion rates in 166 histology and 73 cytology samples were reported as 239 (53.6%). The EGFR mutations were positive in 220 women (23.1%), 98 men (13.5%) and 79 Asian (48.8%) patients. In another study reported by Sandra et al. (14), EGFR exon 19 deletions were present in 53%of 2,142 patients with an early lung adenocarcinoma, while this rate was 61%among patients with an advanced adenocarcinoma. The frequency of EGFR mutations in the NSCLC tumor tissue samples was reported as 19% in men and 26% in women. In a study by Baek et al. (15) involving a Korean population of 1,738 patients, the efficacy of EGFR tyrosine kinase inhibitors in NSCLC patients with exon 21 L858R and exon 19 deletion mutations of the EGFR gene was examined. In the aforementioned study, 88 patients (5.1%) were positive for rare or complex mutations and 54 were treated with tyrosine kinase inhibitors. Rare and complex mutations were identified in 33 and 21 patients, respectively. In another study, Bircan et al. (16) investigated EGFR exon 19 and exon 21 mutations in 25 patients, including 14 with an adenocarcinoma and 11 with a squamous cell carcinoma. The EGFR mutations were identified 11 (44%) patients with NSCLC, while exon 19 and exon 21 mutations were identified in eight (32%) and five (20%) patients, respectively. Two patients had both mutations concomitantly. Of the six (35.7%) patients with adenocarcinomas, three had exon 19 and the remaining three had exon 21 mutations. Of the seven (54.5%) patients with squamous cell carcinomas, five had exon 19 and two had exon 21 mutations. In their study investigating the EGFR mutations in a Chinese sample of 157 individuals, Li et al. (17) detected exon 19 and exon 21 mutations in 22 and 35 individuals, respectively. Quan et al. (18) found that the total rate of somatic EGFR mutations was 48.02% in the Chinese population, including 354 patients with NSCLC. Of those mutations, 27.40% were in exon 19 and 25.99% were in exon 21. The most common mutations in exon 19 and exon 21 were identified as E746-A750del (8.47%) and L858R (10.17%) mutations, respectively. Of all the patients with EGFR mutations, 60.13% were women and 38.81% were men[adjusted odds ratio (OR), 1.93, 95% confidence interval (CI), 1.07–3.51, P:0.029]. The distribution of patients by age groups was 58.62% in the <60 years age interval and 40.67% in ≥60 years age interval (adjusted OR, 1.87; 95% CI, 1.20-2.92; P:0.006). The distribution of the EGFR mutation rates according to histological tissue type was 52.76% for adenocarcinoma and 26.56% for non-adenocarcinoma cases. The mean age of the patients in the study was 62 years.

We found that the rate of the EGFR exon 19 (747–750) deletion mutant genotype was higher among lung cancer patients, and although the frequencies differed, our results are overall consistent with those previous studies (12, 14–18). The mean age of the patient group in the present study was similar to those reported by Li et al. (17) and Quan et al. (18). In the study by Baek et al. (15), the rates of adenocarcinoma and non-adenocarcinoma cases were 88.9 and 11.1%, respectively, while in the study of Bircan et al. (16), these rates were 56% for adenocarcinoma and 44% for squamous cell carcinoma. In the study by Li et al.(17), the rates were as follows: squamous cell carcinomas: 8.3%, adenosquamous carcinoma: 50%, mixed adenocarcinomas with bronchiole alveolar components and bronchiole alveolar adenocarcinomas: 17.6% and other adenocarcinomas: 58.8%. The distribution according to histological tissue types was 81.92% for adenocarcinoma, 16.95% for squamous cell carcinoma and 1.13% for large cell carcinoma in the study by Quan et al. (18). The

distribution of lung cancer cases in terms of the major histological tissue types in the present study was adenocarcinoma, squamous cell carcinoma, and small cell carcinoma, followed by large cell carcinoma. This finding is also consistent with the aforementioned studies, indicating that the male sex, smoking, and old age are greater risk factors for the development of lung cancer. This is the first study, however, to provide proof of the link between old age and the risk of developing lung cancer by presenting statistical analyses based on the distribution of a sample population according to age groups, and secondly, by calculating the ratio of lung cancer patients in each age group.

Conclusion

In conclusion, the EGFR exon-19 747–750 deletion mutant genotype represents a risk factor for the development of lung cancer with individuals with this mutation being 2.64 times more likely to develop the disease. According to our study results, the EGFR exon-19 747–750 deletion mutant genotype frequencies did not differ significantly between the histological tissue types, while smoking, male sex, and old age were found to be the most significant risk factors for the development of lung cancer. Considering the distribution of lung cancer patients across the different age groups, it is evident that older age represents a risk factor for the development of lung cancer. Nonetheless, further large-scale, comprehensive studies are needed to establish a conclusion.

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Author's Contributions: **DYE, EN, RBA, EDE, NE, DDY, EA;** Research concept and design, **EN;** Patient examination, Research the literature, preparation of the article. **NE;** Genetic Analsis**. DYE;** Revision of the article.

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Adrenomedullin has effects on hypoxia-inducible factor 1-alpha in hypoxia in several organs of female rats

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Abstract

Objective: Vascular dysfunction induces impaired O_2 delivery and hypoxia, and adrenomedullin (AdM) have a role in hypoxia and angiogenesis. The reaction of the organism exposed to hypoxia and the effect of AdM over HIF-1α has been investigated in this study.

Material and Methods: Female rats were divided into 4 groups (control, hypoxia, control + AdM and hypoxia + AdM). Hypoxia groups were provided hypoxia containing 10% oxygen and 90% nitrogen for 1 week. AdM treatment groups were injected 1.25 nmol/kg AdM for 4 days intraperitoneally. AdM and HIF-1α levels were measured in heart, lung, and kidney tissues with ELISA.

Results: AdM levels increased in hypoxia + AdM group than hypoxia group, however, was not increased in control + AdM group when compared with the control group in heart tissue. Contrarily, AdM decreased in control + AdM group in the kidney, while increased in control + AdM, and hypoxia + AdM groups in the lung when compared to the control group. The HIF-1α level was higher in control + AdM group in the kidney, and hypoxia + AdM group in the lung. HIF-1α levels in heart tissue were decreased in hypoxia group when compared to the control group.

Conclusion: Tissues respond to hypoxic conditions at different times, and at distinct levels. AdM may be used to induce HIF-1α therapeutically before ischemic conditions.

Key words: Adrenomedullin, hypoxia, hypoxia-inducible factor 1α, angiogenesis

Introduction

Angiogenesis is regulated by hypoxia for supplying adequate oxygen to the cells. The lungs, heart, vascular, and red blood cell system involve in angiogenesis for providing balance against inadequate and excessive oxygenation (1). De novo vessels form in embryogenesis by vasculogenesis, in addition, new blood vessels form in pathological and physiological conditions including tumor neovascularization, ischemia, rheumatoid arthritis, regeneration, wound healing, menstrual cycle in adulthood (2). A hypoxic microenvironment is generated by physical and capillary injury and tumor development. This condition triggers angiogenesis by stimulating angiogenic factors (1).

Hypoxia-inducible factor (HIF) is the key transcriptional regulator of angiogenic pathways including the triggering vascular endothelial growth factor (VEGF) by hypoxia (1). HIF-1 is a DNA binding factor consisting of α and β subunits (3) and binds to 3' enhancer of erythropoietin which is a hormone that regulates vascular oxygen content (3,4).

HIF-1 also regulates other responses to hypoxia including angiogenesis and the activation of several genes for surviving on low oxygen conditions (5).

HIF-1α is inducible by hypoxia and it is rapidly degraded in normoxic conditions in the oxygen-dependent degradation domain by the ubiquitin-proteasome pathway (6). The overexpression and dysregulation of HIF-1α by either genetic alternations or hypoxia have been massively involved in cancer biology and several pathophysiologies, including tumor invasion, energy metabolism, angiogenesis, cell survival, and vascularization (7).

Adrenomedullin (AdM) is a vasodilator peptide and was discovered in a neuroendocrine tumor pheochromocytoma (8). AdM is synthesized in many tissues, however, it is chiefly synthesized in the medulla (9). AdM has a wide range of effects involving inhibition of cardiovascular alteration, the regulation of vascular endothelial function, vasodilatation, decreasing insulin resistance, and adjusting adipogenesis (10). The AdM binding sites were found in the membranes of kidney, lung, and heart (11).

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AdM provides enough oxygen to tissues by expanding the blood vessels as a vasodilator peptide in hypoxia (12). AdM protects against ischemia-reperfusion injury after stroke (9). Furthermore, there are hypoxia response elements in human AdM promoter (9).

The reaction of the organism exposed to hypoxia and the effect of AdM over HIF-1α has been investigated in this study.

Material and Methods

The rats were obtained from Inonu University Laboratory Animal Reproduction and Research Center. Rats were housed in cages at a 12/12-hour light/dark cycle, and the temperature of 22 ± 2°C. All processes with rats were managed in guidelines established by the Institutional Animal Care and Use Committee of the university, Public Health Services Policy, and the Animal Welfare Act.

Twenty-eight 12 months old female Sprague dawley rats were portioned into 4 groups randomly: control (n = 8), hypoxia (n = 7), control + AdM (n = 6) and hypoxia + AdM (n = 7). Hypoxia groups were provided with 10% oxygen (13,14) and 90% nitrogen for 1 week, and the control group was provided normoxia. During hypoxia, AdM groups were treated with 1.25 nmol/kg AdM (Rat Adrenomedullin 24-50, Phoenix Pharmaceuticals, Inc., CA, USA) for last 4 days intraperitoneally.

Rats were anesthesized with 1500 µL/kg ketamine, 500 µL/kg xylazine, then, heart, lung, and kidney tissues were collected. Tissues were homogenized in 2 mM PBS buffer (pH 7.3), and AdM and HIF-1α concentrations were measured by ELISA (Rat Adrenomedullin, ADM ELISA Kit CK-E30105; Rat hypoxia-inducible factor 1α, HIF-1α ELISA Kit CK-E30271, Hangzhou Eastbiopharm Co., Ltd., Zhejiang, China) according to manufacturer's instructions.

The results were expressed as means ± SD. The differences between groups were calculated with One-way ANOVA and LSD posthoc test (IBM SPSS Statistics Version 24), and values smaller than 0.05 were accepted as statistically significant.

Results

AdM level was decreased in the hypoxia group than the control group and hypoxia + AdM group in heart tissue ($p < 0.05$). Furthermore, AdM was higher in control group than hypoxia and control + AdM groups in kidney tissue ($p < 0.05$). Additionally, AdM was increased in all groups when compared with the control group in lung tissue (Table 1, Figure 1, $p < 0.05$).

HIF-1α was lower in hypoxia and hypoxia + AdM groups than the control group in heart tissue ($p < 0.05$). HIF-1α levels of control + AdM group was significantly increased than other groups in kidney tissue ($p < 0.05$). Control and hypoxia group HIF-1α levels were different from hypoxia + AdM and control + AdM groups in lung tissue ($p < 0.05$). Control + AdM and hypoxia + AdM group levels were increased when compared with control and hypoxia groups ($p < 0.05$, Table 2, Figure 2).

Table 1. AdM levels. (Means ± SD)

AdM (ng/L)	Heart	Kidney	Lung
Control	14.4 ± 2.7	16.7 ± 4.2	11.2 ± 1.3
Hypoxia	11.9 ± 1.3	12.9 ± 2.1	13.6 ± 2.1
Hypoxia + AdM	15.5 ± 1.4	15.4 ± 3.3	14.6 ± 2.5
Control + AdM	13.5 ± 2.9	12.8 ± 2.7	15.3 ± 1.6

Table 2. HIF-1α levels. (Means ± SD)

HIF-1α (ng/mL)	Heart	Kidney	Lung
Control	0.84 ± 0.33	0.70 ± 0.10	0.47 ± 0.1
Hypoxia	0.50 ± 0.13	0.78 ± 0.09	0.47 ± 0.05
Hypoxia + AdM	0.61 ± 0.11	0.86 ± 0.20	0.89 ± 0.20
Control + AdM	0.68 ± 0.14	1.03 ± 0.15	0.81 ± 0.28

Discussion

HIF-1α is induced in specific cell types including kidney, brain, liver, heart, skeletal muscle, lung, pancreas, intestine, and additionally, different levels of responses are observed in these specific cells (15–17). The oxygen concentration varies between tissues, for instance, the pO_2 of the kidney is 9.5 ± 2.6%, lung 5.6% (18), and myocardium 10% (19). It is possible that the hypoxia conditions of this study could remain normoxic to heart and kidney tissues. HIF-1α levels of heart, kidney, and lung were different from each other and the responses of these tissues to hypoxia were varied. HIF-1α was increased in hypoxia and hypoxia + AdM groups than the control group in the heart, suggesting that the amplitude of induction of heart is lower than other tissues. In the lung, alveolar cells are oxygenated from inspired air, rather than oxygenation with blood (17). It is possible that the response to hypoxia in the lung may earlier and distinct from other tissues. Although AdM expression is activated by HIF-1α (9), AdM treatment induced HIF-1α in hypoxia + AdM and control + AdM groups in lung. These results show that AdM may induce HIF-1α in the lung.

HIF-1α is found in the nucleus of the brain, heart, kidney, and liver cells in normoxia. The maximal expression of HIF-1α in hypoxia is 5 h, then expression decreases to basal levels in 12 h (16). It is observed in vivo studies that prolonged hypoxia increases HIF-1α mRNA (20). Rats provided hypoxia for 1 week in this study, thus, we could unable to observe peaks in HIF-1α levels. The induction of HIF-1α in kidney and liver is permanent only for 3 hours, then induction is disappeared. Additionally, HIF-1α is induced in more serious hypoxia in the liver and kidney (17). The HIF-1α level in kidney was higher in control + AdM group in this study. It is striking that AdM increased HIF-1α in control group independently from hypoxia.

Studies showed that HIF-1α induces AdM in hypoxic conditions to provide vascularization. Furthermore, AdM is overexpressed in human malignant tumors (21). AdM is elevated in several cardiovascular diseases according to the severity of the disease. Nitric oxide (NO) synthesis is activated by AdM resulting regulation of blood fluid and protective effect in ischemia/reperfusion injury and myocardial ischemia-induced arrhythmias (9).

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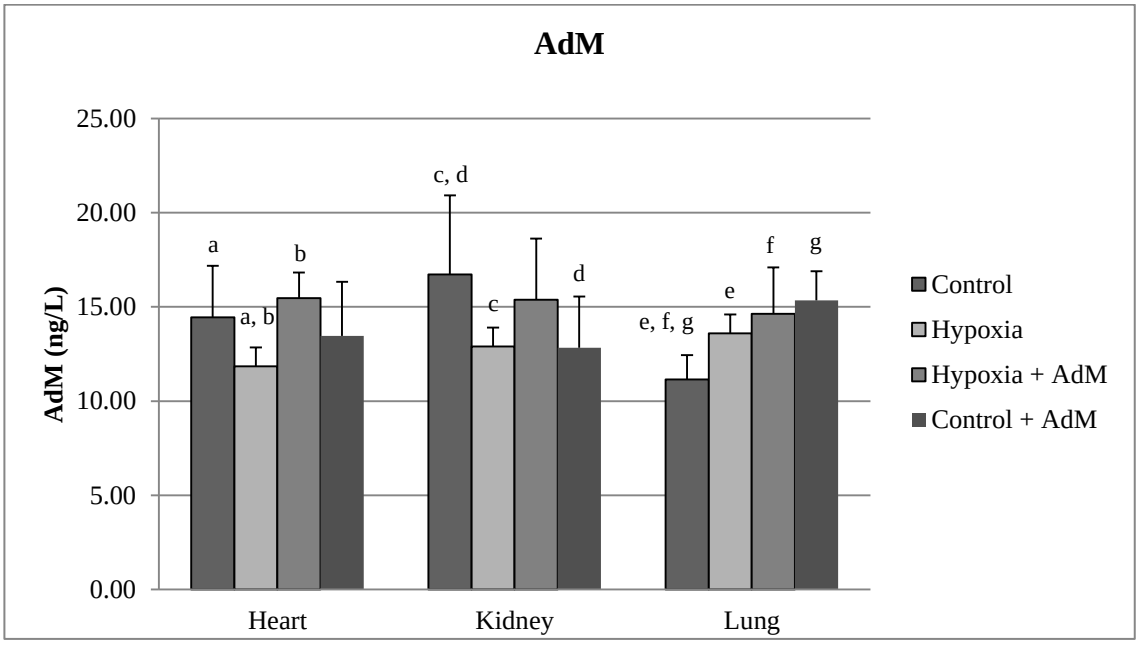

Figure 1. The differences between groups in AdM levels. Levels with same letters indicate significant differences ($p < 0.05$)

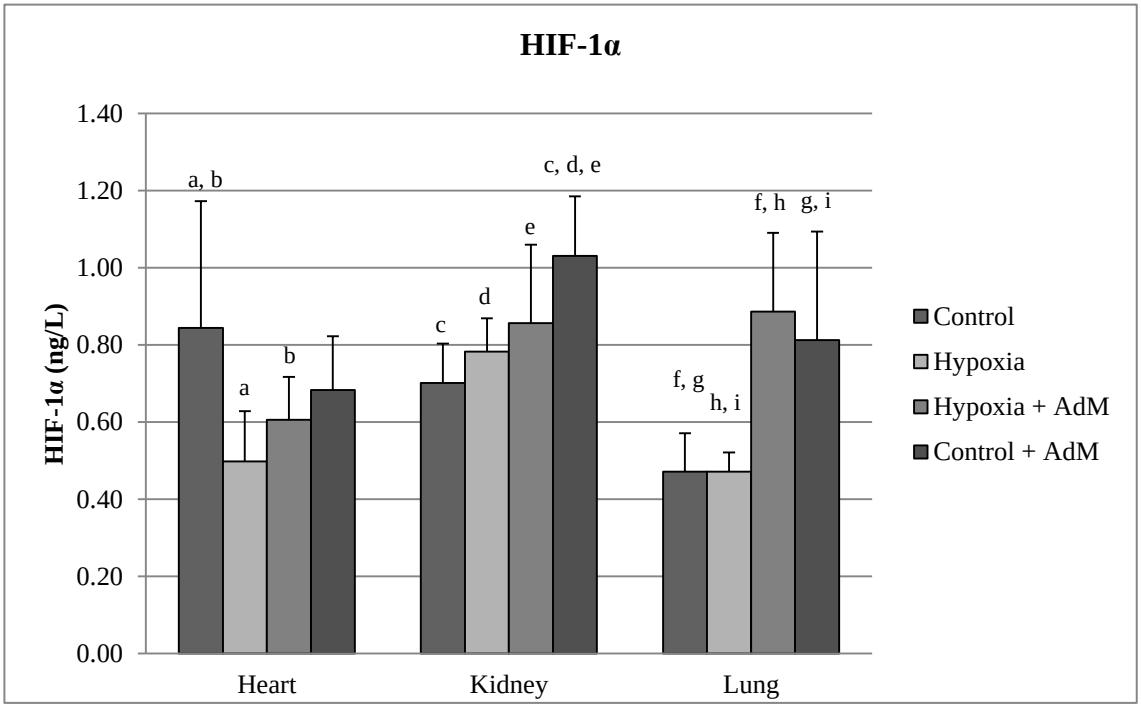

Figure 2. The differences between groups in HIF-1α levels. Levels with same letters indicate significant differences ($p < 0.05$)

In this study, hypoxia decreased AdM levels in hypoxia group in heart tissue; however, AdM treatment increased the level in hypoxia + AdM group. This increase might trigger angiogenesis for supplying enough oxygen to the cells. AdM is a controller of renal function and also have a protective effect in ischemia/reperfusion injury in the kidney (9).

AdM treatment in hypoxia + AdM group kept AdM to control levels in kidney suggesting that AdM decreased in heart and kidney after its contribution to angiogenesis or protection against hypoxia. AdM preserves pulmonary hypertension caused by high blood flow (9). AdM was increased in all groups when compared to control group in the lung in this study.

This may be the result of the sensibility of lung tissue to hypoxia, and AdM treatment. HIF-1α levels induced by hypoxia are the cell type-specific (1).

Sexual dimorphism draws attention, and the National Institutes of Health (NIH) emphasizes the necessity of researching diseases in both sexes (22). Besides, angiogenesis is crucial for females due to the female reproductive system. According to the former studies, hypoxia induces HIF-1α in kidney transiently in male (15), female rats (16), as in our study. Additionally, HIF-1α is upregulated in myocardial endothelial cells, cardiomyocytes (17), and heart in female rats (16), however, we could not detect an increase. Regarding AdM, AdM is a protective molecule against myocardial ischaemia in male rats (23). AdM is expressed averagely strong in the cortex of kidney; lower in lining epithelial cells of lung and myocardium in the male and female rats (24).

Conclusion

Angiogenesis is an important process recently as abnormal, inadequate and excessive vessels are responsible for many pathophysiological conditions including carcinogenesis. Tumor cells generate hypoxic microenvironment and then, angiogenesis is stimulated by hypoxia. Additionally, AdM is synthesized by tumor cells and its expression is stimulated by HIF-1α (9). Occlusive vascular diseases trigger a wide proportion of morbidity and death recently. The HIF pathway regulates initial responses to hypoxia (19); hence HIF may be activated therapeutically before ischemic situations for accelerating response to hypoxia (1), ameliorating ischemic vascular diseases such as atherosclerosis (19) and regulating angiogenesis to prevent vascular dysfunctions.

Promoting angiogenesis causes tumor growth; however, it would be significant in ischemic conditions. According to the results, AdM treatment may provide a rapid and continuous adjustment to hypoxia, and, AdM may be a part of therapeutic vasculogenesis. Our study showed that AdM induces HIF-1α, thereby; AdM may be used to induce HIF-1α therapeutically before ischemic conditions. We suggest that AdM could regulate HIF pathway that is a potential therapeutic target for vascular dysfunctions including neovascular eye disease, peripheral artery disease, and cancer. Further studies about the role of AdM in Notch signalling pathway would be useful before therapeutic adjustments. Additionally, the determination of VEGF that induced by AdM and HIF-1α will be a guide for the dose of AdM.

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Author's Contributions: **AAC, MY**; Research concept and design, Animal studies, Research the literature, preparation of the article. Chemical Analysis**. AAC;** Revision of the article.

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Prognostic factors affecting local recurrence and systemic metastasis in early stage breast cancer

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Abstract

Objective: Breast cancer is the most common cancer in women. Mortality in breast cancer is associated with recurrence and metastases. The aim of this study was to determine the causes of local recurrence and metastasis in operated early stage breast cancer.

Material and Methods: The files of the patients diagnosed with operated early stage breast cancer followed up at Dicle University Medical Faculty Medical Oncology Department between 2001-2010 were analyzed retrospectively. The group of patients with recurrence was referred to as the relapse-metastasis group and the other patients were called the remission group. Between these two groups, age, sex, tumor histology, size, presence of lymphovascular invasion, tumor grade molecular properties such as, ER, PR, HER, staging studies; time characteristics, surgical characteristics, time between surgery-chemotherapy and surgery-radiotherapy; The relapse-metastasis group and the remission group were compared in terms of chemotherapy. SPSS 22 was used for statistical analysis. $p<0.05$ was considered significant.

Results: 479 patients were included in the study. The number of patients in the remission and relapse-metastasis group was 343 and 136, respectively. Tumor diameter was 3.33 cm in the remission group and 4.58 cm in the relapse-metastasis group, and the difference was statistically significant ($p<0.01$). The percentage of involvement of more than 4 lymph nodes were 30.45% and 55.7% in the remission and relapse-metastasis groups, respectively ($p <0.01$). The percentage of the lymphovascular invasions were remission group and relapse-metastasis group 46.4% and 80% respectively. The percentage of patients with surgery-chemotherapy time> 1 month was 9.7% and 24% in the remission and relapse-metastasis group, respectively. In remission group 30.9%; relapse-metastasis group 47.7% of the patients had incomplete staging at the time of diagnosis.

Conclusion: The factors determining the recurrence in patients with operated early stage breast cancer were tumor size, number of lymph node involvement, incomplete staging at the time of diagnosis, start chemotherapy later than 1 month.

Keywords: early stage breast cancer, recurrence, metastasis

Introduction

Breast cancer is a major health problem for women worldwide. One in 8 women gets breast cancer during their lifetime. It is the most common cancer in women in the United States; is the second leading cause of cancer - related deaths. According to annual cancer statistics, 26% of new cancer cases are breast cancer; it is estimated that 15% of cancer-related deaths will be breast cancer (1,2).

Early diagnostic procedures, adjuvant chemotherapy and hormonal therapy reduced mortality in breast cancer (3). As in the world, the most common cancer in women in our country is breast cancer (4).

Recurrence and metastases are the cause of mortality in early stage breast cancer (5).

Tumor sizes, poor histologic structure, lymphovascular invasion at diagnosis, local advanced diseases were known features for recurrence-metastasis (6).

Factors other than these risk factors have not been fully clarified in the literature.

The aim of this study was to determine the causes of local recurrence and metastasis in operated early stage breast cancer.

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Material and Methods

The files of breast cancer patients admitted to Dicle University Medical Faculty Hospital Medical Oncology Department between 2001 and 2010 were reviewed retrospectively. Patients with metastasis at the first diagnosis were excluded. Operated patients were included in the study. Relapse-metastasis group was defined as relapse patients during follow-up and remission group was called as other patients. Between these two groups, age, sex, histology of tumor, tumor size, presence of positive lymph node, lymphovascular invasion, ER, PR, HER status were recorded. Staging studies at the time of diagnosis (such as chest radiography, abdominal USG, scintigraphy, tomography), surgical characteristics (surgical site, mode of operation), time of adjuvant treatments (time(month) between surgery-chemotherapy and surgery-radiotherapy relapse-metastasis group and remission group were compared.

Statistical analyses were performed using the SPSS software version 21. Variables were expressed as means ± standard deviations (SDs), and categorical variables were expressed as counts and percentages. The chi-square test or Fisher's exact test was used to compare these proportions in different groups. The results were evaluated with 95% confidence interval and p <0.05 significance level.

Results

After screening, 479 patients were included in the study. In the remission and relapse-metastasis group there were 343 and 136 patients, respectively. The mean age in the remission group was 48.7 years (18-79), and the mean age in the relapse-metastasis group was 47.9 years (23-77) (p> 0.05). The most common histological structure was ductal adeno-ca in both groups. Most patients in the recurrence-metastasis group had ductal histology and were statistically nearly significant (p:0.061).

Tumor diameter was 3.33 cm in the remission group and 4.58 cm in the relapse-metastasis group, and the difference was statistically significant (p<0.01).

The tumor diameter was T2 (2-5 cm) in most patients. There were 234 patients (73.8%) in the remission group and 83 patients (26.2%) in the relapse-metastasis group.

There were a significant difference between the two groups in terms of tumor diameter (p <0.01). The tumor diameter was greater in the relapse-metastasis group (p <0.01).The percentage of N0 patients in our study was 39% in the remission group; and 14% in the relapse-metastasis group.

The percentage of involvement of more than 4 lymph nodes were 30.45% and 55.7% in the remission and relapse-metastasis groups, respectively (p <0.01). The percentage of lymphovascular invasion remission group and relapse-metastasis group were 46.4%, 80% respectively (Table 1).

There was no statistically significant difference between ER, PR and HER between the two groups. When the patients were analyzed in terms of the mode of operation, it was seen that 90.2% of the patients who developed recurrence-metastasis underwent MRM.

It was observed that breast conserving surgery was performed mostly in university hospitals (40%). The percentage of patients with surgery-chemotherapy time> 1 month was 9.7% and 24% in the remission and relapse-metastasis group, respectively.

Statistically significant difference was found between the two groups. In remission group 30.9%; recurrence-metastasis group 47.7% of the patients had incomplete staging at the time of diagnosis(Table 1).

	Remission group	Relapse-metastasis group	p value
n: 479	343	136	
Gender (F/M)	341/2	133/3	
Mean age (years)	48.7(18-79)	47.9(23-77)	>0.05
Ductal carcinoma	81.2%	87.3%	>0.05
Tumor diameter (cm)	3.3	4.58	<0.01
T2 (2-5cm) tumor	73.8%	26.2%	<0.01
Lymphovascular invasion	46.4%	80%	<0.01
Percentageof NO patients	39%	14%	<0.01
>4 lymphnode involvement	30.4%	55.7%	<0.01
Modified radicalmastectomy	80.4%	90.2%	<0.01
Surgery-chemotheraphy time (>1 month)	9.7%	24%	<0.01
Incomplete staging in initial diagnosis	30.9%	47.7%	<0.01

Discussion

In this study, the most important finding is that in the breast cancer incomplete staging at the time of diagnosis, start chemotherapy later than 1 month were found to be associated with recurrence.

Breast cancer is the main health problem for women in the world. The most common cancer in women in our country is breast cancer. Local recurrence and distant metastasis in breast cancer are associated with mortality. Due to the increased scans, most patients are diagnosed at an early stage. Chemotherapy and hormonal therapy in breast cancer reduces recurrence and mortality (7).

The larger tumor in breast cancer, worsens the prognosis. There is a direct correlation between tumor size and recurrence and distant metastasis. In a study by Rosen et al. which examined the relationship between 20-year tumor size and recurrence-metastasis survival, disease-free survival was shortened as tumor size increased (8). In our study, tumor size was found to be larger in the relapse-metastasis group.

Survival rates of those with tumors less than two centimeters are high. Carter and colleagues found that tumor size was an independent prognostic factor for survival in the study of 24,740 breast cancer patients. The 5-year survival rate was 96.3% in patients with tumor size less than 2 cm; in patients with a tumor size of 5 cm and over, this rate was 45.5% (9).

In retrospective prognostic index determination study performed by Galea et al. in 387 breast cancer patients, tumor size was found to be a prognostic factor (10). In our study, mean tumor diameter in the remission group was 3.33 cm and 4.58 cm in the relapse-metastasis group and statistically significant. In our study, the percentage of patients with tumor diameter 2 cm and over was 88.2% in the relapse-metastasis group; whereas in the remission group this rate was 76% and statistically significant ($p < 0.001$). Consistent with the literature, in our study, the recurrence-metastasis rate increased as the tumor diameter increased.

The number of metastatic lymph nodes is also a factor that affects the prognosis negatively. In patients with one to three lymph node positive patients, recurrence rate is lower than in patients with four or more lymph node positive patients and the likelihood of long-term survival is higher. The 5-year survival rate in nod negative patients is 70-80%, while the 5- year recurrence rate is approximately 20%. In the American National Cancer Institute's Surveillance Epidemiology and End Results (SEER) program, 24740 patients were examined and it was determined that as the lymph node involvement increased, the likelihood of recurrence increased and survival decreased and this correlation was found to be directly proportional to the increase in tumor size (9). The percentage of N0 patients in our study was 39% in the remission group; and 14% in the relapse-metastasis group. The percentage of patients with the number of involvement nodes 4 or more was 30.4% in the remission group and 55.7% in there relapse-metastasis group and was statistically significant.

The recurrent-metastasis rate increased as the number of involvement nodes increased.

Adjuvant chemotherapy usually begins 4-6 weeks after the operation. The benefit of starting earlier has not been shown; however, it has been reported that it is harmful to start late than 12 weeks. In the retrospective analysis of Danish breast cancer group with 5003 patients, it was found that starting from the first 3 weeks in terms of survival analysis grouped as first three weeks, 1-3 months and after 3 months, according to chemotherapy patients, it did not provide additional benefit; delay after three months was found to have a negative effect on survival (11). In our study, the time to start chemotherapy was designed as 1 month and before or after 1 month. In the remission group, 90.3% of patients who received early chemotherapy; it was found to be 76% in the relapse-metastasis group and was statistically significant. Most of the patients in the remission group had chemotherapy started early.

Currently, surgical treatment of breast cancer includes breast conserving surgery and modified radical mastectomy (12). When the patients were evaluated in terms of operation type, breast conserving surgery was performed mostly in the remission group; modified radical mastectomy was performed in patients in the relapse-metastasis group. There was a statistically significant difference between the mode of operation and recurrence. Recurrence was more common in MRM patients. This may be related to the progression of relapse patients at the time of diagnosis. The limitations of our study were retrospective design.

Conclusion

The factors determining the recurrence in patients with operated early stage breast cancer were tumor size, number of lymph node involvement, incomplete staging at the time of diagnosis, start chemotherapy later than 1 month.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards

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: AI: Research concept, Design and Revision; ZP: Data collecting, Preparation of article, and Revisions. All authors approved the final version of the manuscript

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Acquired von Willebrand disease in chronic myeloproliferative disorders: a prospective single-center study

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Abstract

Objective: The rate of acquired von Willebrand disease (aVWD) among myeloproliferative patients is substantial enough to merit serious consideration, as it is thought to play a role in hemorrhage. We aimed to investigate the rate of acquired von Willebrand disease (aVWD) in chronic myeloproliferative disorders (MPD)

Materials and Methods: The present study was conducted prospectively on 70 patients admitted to hematology clinic. Complete blood count, PT, aPTT, vWF:Ag level, vWF:RCoF test, and factor VIII levels were analyzed for all patients. A finding of vWF:RCoF / Ag < 0.7 was accepted as predisposition to aVWD.

Results: Of the patients, 33 (47.1%) were male, 37 (52.8%) were female, and the mean age was 50 ± 16.25. We detected aVWD in 19 (vWF:RCoF / Ag test < 0.7) (28%) of the 70 patients in the study group. Predisposition to aVWF was present in 7 of the 16 patients in the ET group(43.7%), in 4 of the 11 PV patients (36%), and in 8 of the 43 CML patients (18.6%). There was no statistically significant difference in the presence of aVWD between the three disease groups (p: 0.079).

Conclusion: The underlying mechanism of aVWD is still not fully resolved. Myeloproliferative diseases are one of the few diseases that can cause avWS. It should be kept in mind that aVWD may play a role in pathogenesis in people with chronic myeloproliferative disease, especially in cases of hemorrhage occurring in ET and PV patients.

Keywords: Acquired von Willebrand disease, chronic myeloproliferative disorders, hemorrhage

Introduction

Acquired von Willebrand disease (aVWD) is a bleeding diathesis disorder associated with von Willebrand factor (VWF) deficiency or functional failure as a result of underlying disease. These underlying diseases have been found to be primarily monoclonal gammopathies, lymphoproliferative diseases, myeloproliferative diseases, autoimmune disorders, solid tumors, infectious diseases, heart valve diseases, or other unidentifiable conditions (1). People diagnosed with aVWD do not have a family history of the disease and have complaints of bleeding that develops subsequent to other conditions. The type of underlying disease is closely related to the severity of VWF deficiency and the general condition of the patient (2).

Chronic myeloproliferative disorders (CMD) such as polycythemia vera (PV), essential thrombocytosis (ET), and chronic myeloid leukemia (CML) may co-occur with aVWD, although such cases are rare. In aVWD, which develops secondary to chronic myeloproliferative disorders, critical deficiencies in the molecular structure of VWF are seen; this is thought to cause bleeding problems (3). CMDs are characterized by normal factor VIII (FVIII) and VWF antigen (VWF:Ag) levels; however, ristocetin cofactor activity (VWF:RCoF) and collagen binding activity (VWF:CBA) are decreased. The increased cell burden in CMD patients predisposes them to aVWD. In CMD, the normally large multimeric and functional VWF structure is either absorbed by malignant of hyperproliferative cells or undergoes proteolysis. The result is the development of VWF structures separated into small fragments that are less functional in the hemostatic system (4).

CMD is also implicated in a number of developing antibodies as well as increased clearance and proteolysis resulting from degeneration in the VWF structure. In people with aVWD, the structure of VWF in the multimeric structure has been observed to decrease.

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These antibodies increase the breakdown of large VWF multimers in circulation or lead to proteolysis by making them more sensitive. This situation is related to VWF production and the reduction in binding of the VWF produced to cells. As a result of this extensive paucity of production, malfunctioning, and proteolysis, aVWD develops (5). This is also encountered in cases of clonal lymphoproliferative and autoimmune diseases (e.g., systemic lupus erythematosus). Although laboratory findings resemble the hereditary type of von Willebrand disease, the absence of bleeding abnormalities in patients' pasts and the lack of a family history support a diagnosis of aVWD (6).

Only a few studies have reported on the association of aVWD with myeloproliferative diseases. In this study, we aimed to identify the presence of acquired von Willebrand disease in patients diagnosed with chronic myeloproliferative disease.

Materials and methods

This single-center study was conducted prospectively at the department of hematology. Patients diagnosed with CMD who agreed to participate were recruited into the study. Informed consent was obtained in writing from all participants. Ethical approval was granted by medical faculty clinical research ethics board (07/03/2017, decision no: 002). The present study was supported by the university scientific research projects department (SRP). Patients diagnosed with bleeding disorders prior to CMD diagnosis were not included in the study. Complete blood count (CBC), prothrombin time (PT), active partial thromboplastin time (aPTT), VWF:Ag level, VWF:RCoF test, and FVIII levels were analyzed for all patients. A finding of VWF:RCoF / Ag < 0.7 was accepted as predisposition to aVWD.

For complete blood count, venous blood was placed in tubes containing ethylenetetraacetic acid and analyzed using an Abbot Cell-Dyn 3700 automated blood count machine. For analysis of VWF:Ag, PT, and aPTT levels, venous blood samples were placed into tubes containing sodium citrate. STA-Compact and STA-R Evolution brand autoanalyzer commercial kits were used to test VWF:Ag and FVIII levels. PT and aPTT levels were analyzed using the Dade Behring BCS XP system. Ristocetin cofactor levels were tested using the ELISA method and the Helena AggRAM analyzer.

Statistical Method: Descriptive statistics for continuous variables in the study were expressed as mean, standard deviation, minimum, and maximum values, while categorical variables were expressed as number and percentage. One-way analysis of variance (ANOVA) was performed to compare group averages in terms of continuous variables. Following analysis of variance, the Duncan test was used to identify the different groups. Pearson correlation coefficients were calculated separately for the groups in determining the relationship between these variables. The Chi-square test was conducted to determine the relationship between groups and categorical variables. A p value lower than 0,05 was accepted as statistically significant, and the SPSS statistical package program (version 20.0) was used for the calculations.

Results

The median age of all 70 patients studied was 51.18 ± 16.47. Of the patients, 33 (47.2%) were male (mean age 50.67 ± 15.13) and 37 (52.8%) were female (mean age 49.56 ± 17.31). Sixteen (22.8%) of the patients had a diagnosis of ET, 11 (15.8%) had PV, and 43 (61.4%) had CML. No significant difference in age with respect to the type of diagnosis was found (p: 0.082). There was no statistically significant difference in age between the sexes when mean ages of all patients were compared on the basis of gender (p: 0.79) (Table I). In terms of gender distribution, no statistically significant difference between the overall disease groups was observed (p = .498) except PV group that number of female patients were higher (p: 0.009). In the present study, predisposition to aVWD was found in 7/16 (43.7%) of the ET patients, in 4/11 of the PV patients (36.3%), and in 8/43 of the patients with CML (18.6%). There was no statistically significant difference between the three disease groups with regard to the presence of aVWD (p: 0.079). The distribution of the red blood cell numbers(RBCs), hemoglobin levels, platelets numbers, mean corpuscular volume (MCV), and hematocrit values in the patients hemogram samples were evaluated for all three disease groups (PV, ET, and CML). RBCs were significantly higher in PV patients than in ET and CML patients (p: 0.01). Hemoglobin values were significantly higher in PV patients compared with CML patients (p: 0.004). The difference in hematocrit levels between the three disease groups was also significant; the highest hematocrit value was found in PV and the lowest value was observed in CML patients (p: 0.001). Platelets values were higher in ET patients than in PV and CML (p: 0.001), significantly. Similar MCV values were obtained for all three groups (p: 0.320).

Values for VWF:Ag, VWF:RCoF, RCoF/VWF (ratio), aPTT and FVIII (%), are showed in Table II. The VWF:RcoF was significantly lower in ET and PV than in CML patients (p: 0.009). Values for aPTT were significantly higher in the ET and PV groups compared with CML (p: 0.001). For other values, no significant difference between the disease groups was found (p< 0.05). Correlation relationship between VWF:Ag, aPTT, FVIII (%), RCoF, RCoF/VWF ratio measurements and hemogram parameters values are shown in Table III. There was a statistically significant negative correlation between platelets levels and RCoF measurements (p< 0.01). There was a significant positive correlation between VWF:Ag and FVIII (%) (p< 0.001), and a significant negative correlation was found between VWF:Ag and RCoF/VWF (p< 0.01). The negative correlation between the RCoF/VWF:Ag ratio and FVIII was statistically significant (p< 0.05). A significant negative correlation was observed between FVIII and RBCs, aPTT, and RCoF/VWF:Ag measurements, the positive correlation between FVIII and VWF:Ag was significant (p: 0.01). There was no significant correlation between FVIII and hemoglobin, hematocrit, platelets, and RCoF measurements (p> 0.05).

Table 1. Patients characteristics and distribution of disease subtype to gender and age

Parameters	Number of patients, (%)	Mean Age ± SD	Range	*P* value
CMD patients, n	70	50.18 ± 16.47	23 - 86	0.082
ET	16 (22.8)	53.19 ± 17.40	23 - 77	
PV	11 (15.8)	58.45 ± 8.75	24 - 86	
CML	43 (61.4)	47.07 ± 16.63	40 - 68	
Gender				0.79
Male	33 (47.2)	50.67 ± 15.13	23 - 86	
Female	37 (52.8)	49.56 ± 17.31	26 - 86	

CMD: Chronic Myeloproliferative Disorders, PV: Polycythemia Vera, ET: Essential Thrombocytosis, CML: Chronic Myeloid Leukemia

Table 2. Analysis of VWF:Ag, VWF:RCoF, RCoF/VWF, aPTT, FVIII (%) values by disease group

Parameters	Mean	St. Dev.	Min- Max.	p value
Von Willebrand Ag (%)				0.57
ET	116.06	71.767	31 - 328	
PV	123.00	58.346	47 - 253	
CML	116.06	49.377	20 - 258	
FVIII (%)				0.76
ET	80.75	55.213	15 - 254	
PV	70.36	43.470	4 - 156	
CML	81.49	41.838	27 - 242	
Von Willebrand ristocetin cofactor (%)				**0.009**
ET	78.90a*	35.01	33 - 177	
PV	72.54a*	23.05	46 - 126	
CML	102.30b*	35.27	44 - 232	
Ristocetin cofactor / von Willebrand Ag (%)				0.078
ET	0.808	0.401	0.32 - 1.99	
PV	0.729	0.448	0.27 - 1.86	
CML	1.313	1.160	0.33 - 6.26	
Activated partial thromboplastin time (s)				**0.001**
ET	34.44a*	4.052	25.9 - 40.3	
PV	33.49a*	2.767	29.9 - 38.8	
CML	30.37b*	1.850	26.4 - 33.8	

PV: Polycythemia Vera, ET: Essential Thrombocytosis, CML: Chronic Myeloid Leukemia ***There is a significant difference between patient groups with different letters, but no significant difference between groups assigned the same letter.***

Table 3. Correlation relationship between VWF:Ag, aPTT, FVIII (%), RCoF, RCoF/VWF ratio measurements and hemogram parameters

Parameters	r
Platelets - aPTT	0.427**
Platelets - VWF:Ag	0.015
Platelets - FVIII	-0.062
Platelets - RCoF	-0.383**
Platelets - RCoF/VWF:Ag	-0.210
VWF:Ag - aPTT	-0.13
VWF:Ag - FVIII	0.630**
VWF:Ag - RCoF	0.154
VWF:Ag - RCoF/VWF:Ag	-0.549**
RCoF/VWF:Ag - WBCs	-0.15
RCoF/VWF:Ag - RBCs	-0.16
RCoF/VWF:Ag - hemoglobin	-0.15
RCoF/VWF:Ag - hematocrit	-0.19
RCoF/VWF:Ag - aPTT	-0.072
RCoF/VWF:Ag - FVIII	-0.243*
FVIII - WBCs	-0.15
FVIII - RBCs	-0.243*
FVIII - aPTT	-0.374**
FVIII - RCoF	0.161

**: $p < .01$, *: $p < .05$, *r: correlation coefficient*

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Discussion

The von Willebrand factor is a factor in the construction of large multimeric glycoproteins, which have two important functions in the hemostatic process. The first of these is to function as a bridge for thrombocyte adhesion in places where there is vascular damage, while the second is to support thrombocyte aggregation. At the same time, VWF also contributes to hemostasis by transporting FVIII in the circulation and prolonging its half-life (7). Acquired von Willebrand disease is a bleeding diathesis caused by a deficiency of VWF or functional impairment due to disease. Although rare, aVWD may be associated with PV, ET, and CML. The most common subtype of aVWD in myeloproliferative disorders is ET (8). Tests for routine VWF deficiency should be performed in such cases. These tests are VWF:Ag, VWF activity, VWF:RCoF, multimer analysis, and collagen binding activity tests (9). In the present study, patients were evaluated by comparing the ratios calculated from VWF:Ag and VWF: RcoF test values and other parameters.

The VWF multimers test is considered the gold standard for diagnosing aVWD. When VWF:RCoF/Ag or VWF:CB/Ag values are at normal levels, the quality of this test as an indicator is enhanced. Under normal conditions, VWF:RCoF and VWF:Ag are in proportion; in this case their ratios are generally accepted as "1". However, change in the multimeric structure results in a decrease to levels below 0.6 or 0.7 due to inhibitors that could not be completely immobilized, thus leading to aVWD (5). Castaman et al. conducted a study of 10 PV, 11 ET, and 8 CML patients. For PV patients they reported the following mean values: platelets were 568×103/μL, VWF:Ag was 167%, VWF:RCoF was 103%, and a VWF: RcoF/Ag ratio was of 0.66. The mean platelet for ET patients were 997×103/μL, VWF:Ag was 138%, VWF:RcoF was 85%, and the VWF:RcoF/Ag ratio was 0.61 (10). Their findings for CML patients were a mean platelets of 334×103/μL, VWF:Ag 248%, VWF:RcoF 157%, and the VWF:RcoF/Ag ratio was 0.64 (10). In our study, for patients diagnosed with PV (11 cases), the mean platelets were 337×103/μL, VWF:Ag was 110%, VWF:RCoF was 72%, and VWF:RcoF/Ag was 0.77. The mean platelets for our patients with ET (16 cases) were 572×103/μL, VWF:Ag was 116%, VWF:RCoF was 78%, and the VWF:RcoF/Ag ratio was 0.80, while those values for patients with CML (43 cases) were 222×103/μL, 104%, 102%, and 1.31, respectively.

According to our findings, an increase in platelet counts showed a significant negative correlation with VWF:RCoF levels ($p< 0.01$). The VWF:RcoF/Ag ratios were 0.77 and 0.80 for the PV and ET patient groups, respectively. Although not to a statistically significant extent, the ratios were found to negatively correlate with the platelets and RBCs parameters. These findings are similar to those of Castaman et al. (10), Franchini et al. (11), Fabris et al. (12), and Tatewaki et al. (13). In their study of 142 PV patients, Mital et al. determined that VWF:RcoF/Ag ratios supported a diagnosis of aVWD in 17 (12%) patients (VWF:RcoF/Ag ratio < 0.7) (3).

In the present study we found that 4 of the 11 PV patients (36%) had VWF:RcoF/Ag ratios suggesting aVWD. According to a study by Mital et al., of 160 ET patients (mean platelets 700×103/μL), 32 patients (20%) had a mean VWF:RcoF/Ag ratio of 0.62 (4). In patients with low VWF:RcoF/Ag, FVIII levels were found to be considerably lower than normal ($p< 0.01$) (4). In the present study, the mean VWF:RcoF/Ag ratio was 0.52 in 7 out of 16 (43%) ET patients (mean platelets 781×103/μL). Seven patients with low VWF:RcoF/Ag in our study were also found to have significantly lower FVIII levels than normal ($p< 0.05$).

In our literature search on CML, we found case studies but not any studies consisting of large patient groups. Of the 43 CML patients in our study, 8 (18.6%) had a VWF:RcoF/Ag ratio of less than 0.7 (mean: 0.48). For those 8 patients, the mean platelets were 237×103/μL, RBCs were 4.69×106/μL, and WBCs were 6.81×103/μL. The reason that these values were normal, however, because those patients were being treated with drugs. In spite of their normal hemogram values, the fact that a VWF:RcoF/Ag ratio < 0.7 was detected in 18.6% of this patient group suggests that not only the number of cells but also different mechanisms had an effect on the current condition of the CML patients. We believe that the details of these mechanisms could be revealed by further studies incorporating greater numbers of patients and more advanced tests.

Acquired von Willebrand disease is frequently reported in ET patient groups (5-30%) (14). In our study, aVWD (defined as VWF:RCoF/Ag < 0.7) frequency was 43% in the ET patient. The fact that similar results have been reported in other studies indicates that newly developing aVWD should be considered in ET patients with complaints of bleeding, except in disorders of plateletes function or drug-induced diseases. ADAMTS13 activity has been shown to increase the clearing of von Willebrand multimers proportional to the increase in platelets count, especially in ET patients (15, 16).

Although the situation in PV patients is not as clear as that in ET patients, the pathophysiology is thought to be similar to that of ET, and includes multimer deficiencies, absorption by over-produced cells, and specific and non-specific antibody systems. The possibility of aVWD as the underlying pathology should be considered, although it is less common in bleeding cases in PV patients than in cases of ET.

As a result of our findings, we determined the presence of aVWD based on low VWF:RCoF/Ag ratios in patients with CMD. However, we should note the weaknesses of the present study. The first is the fact that due to the inability to analyze VWF:CB at the medical center where we conducted our study, the VWF:CB/vWF:Ag ratio could not be checked; this test provides confirmation of the VWF:RCoF/Ag test, thus eliminating the margin of error. The second weakness is that the VWF multimer structure could not be analyzed. This test, by identifying a reduction in the multimeric structure, represents the gold standard for aVWD diagnosis, as it may help to confirm aVWD even in cases where the VWF:RCoF/Ag ratio is normal (> 0.7).

Conclusion

This study aimed to determine the presence of von Willebrand disease in patients with chronic myeloproliferative diseases. Acquired von Willebrand disease (VWF:RcoF/Ag < 0.7) was detected in 19 (28%) of the 70 patients in the study group. A value of 0.7 or greater indicating a normal VWF:RcoF/Ag ratio was in accordance with the literature. Eight of the 43 CML patients (18.6%) in the present study were diagnosed with aVWD, but due to insufficient data on this patient group in the literature, no comparisons could be made with our findings. In light of our study, in CML, aVWD may also develop by means of a mechanism similar to other chronic myeloproliferative diseases. We believe that this pathogenesis can be better elucidated through further studies with more patients. Underlying acquired von Willebrand disease should be considered in cases of acquired bleeding occurring in patients with chronic myeloproliferative disorders.

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Ethical issues: All Authors declare that originality of research/article etc... and ethical approval of research, and responsibilities of research against local ethics commission are under the Authors responsibilities. The study was conducted due to defined rules by the Local Ethics Commission guidelines and audits.

Author's Contributions: Aslanboga M and **Ekinci O**, analyzes and interpretation of the data, preparation of the manuscript, application of the statistical analyses.

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Investigating of the demographic, socioeconomic, and obstetric risk factors of term intrauterine stillbirth cases

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Abstract

Objective: To establish the prevalence, etiology, demographic, socioeconomic, and obstetric risks factors of intrauterine fetal deaths among term pregnancies with no risk factors. Our study is the first to investigate term stillbirth risk factors in such a large population.

Material and Methods: A total of 96 cases of stillbirth between 37th and 42nd weeks with no risk factors out of 90,557 births conducted in 2011-2015 were investigated retrospectively. Eighty patients that had stillbirth in our clinic were chosen as the study cases and 80 others that had risk-free live birth at the weeks of 37-42 chosen randomly accepted as the control group. Variables such as age of mothers, gravidas, parities, level of education of mothers, time since the previous pregnancy, BMIs, weight gained during pregnancy, gestational week, birth weights of infants, systolic and diastolic blood pressures, hemoglobin values, blood glucose levels, white blood cell counts, smoking history, follow-ups at the hospital, gender of babies, and seasonal distribution of stillbirths were evaluated.

Results: The stillbirth rate was found as 14 per million and stillbirth in risk-free population at 37-42 weeks was 1.05 per mill. BMI, hemoglobin levels, and systolic blood pressures of mothers were significantly higher in stillbirths. Any statistically significant difference in mean maternal age, gravida/parity, education level, weight gained during pregnancy, smoking and fetal gender distribution was not established between the groups

Conclusion: Term stillbirths in the risk-free group may be correlated with advanced gestational week, increased BMI, systolic blood pressure, and hemoglobin levels of the mother also insufficient antenatal follow-up.

Keywords: stillbirth, antenatal follow-up, intrauterine exitus, risk factors

Introduction

Worldwide stillbirth rate was shown as 18.4 per million. While this rate and its causes may manifest differences according to countries and even to various regions in a given country, it is observed more in developing countries compared to developed countries (1). Stillbirth rates were established as 0.2% in developed countries, 0.7% in developing countries, and as 2% in South Africa and in certain countries in Asia (2).

Worldwide stillbirth rate was found as 18.4 per mill in 2015, and while the said rate appears to be promising when compared with 18.9 in 2009 and 19.4 in 2000, there is still a need for enlightenment and precautions (3). According to the WHO data, there were 2.6 million stillbirth cases in 2015, and 98% of these cases have been observed in developing or backward countries (3).

ENAP (Every Newborn Action Plan) pulls the 2030 stillbirth rate target down to 12 per mill. In Turkey, stillbirth rate is 9 per mill according to TNS 2013 data.

Such causes of fetal death as syphilis, RH isoimmunisation, preeclampsia-hypertension, and diabetes complicate pregnancy gradually less thanks to antenatal care and treatment. However, intrauterine infections, lethal malformations, chromosomal anomalies, fetal growth retardation, and ablation placentae still cause fetal death in numerous pregnancies (4).

While the determination of causes found in the etiology of stillbirths has shown an increase, one of the factors that would be the cause of death may not always be established in intrauterine deaths (5).

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Rate of stillbirth with no known cause among all still births ranges is between 12% and 50% in the literature (6-9).

The aim of this study is to establish the prevalence, etiology, demographic, socioeconomic, and obstetric risks factors of intrauterine fetal deaths among pregnancies between 37th and 42nd weeks with no risk factors, to determine preventable risk factors, hence, to foresee the precautions necessary to be taken and to increase the healthy mother and infant rate. Our study is the first to investigate term stillbirth risk factors in such a large population.

Material and Methods

A total of 96 cases of stillbirth between 37th and 42nd weeks with no risk factors out of 90,557 births conducted in Zekai Tahir Burak Gynaecology and Obstetrics Training and Research Hospital in 2011-2015 were investigated retrospectively. Patients with systemic diseases, obstetric complications, and previous uterine surgery were excluded from the study. Of these cases, 16 were excluded from the study due to insufficient, but 80 were included in the study.

All the information on cases were collected from computer records, record books, and patient files. Eighty patients that had stillbirth in our clinic were chosen as the study cases and 80 others that had risk-free live birth at the weeks of 37-42 and chosen randomly were accepted as the control group. All the cases that had absent fetal cardiac activity determined by ultrasonography had been established by the records.

In selecting the cases, attention was paid in cases being in the age range of 17-43, last menstrual date of gestational week and above 37 weeks based on ultrasonography, and the absence of fetal cardiac activity (by USG), cases that had chronic diseases (such as diabetes, hypertension, goitre, asthma), thrombophilia, uterine anomaly and previous uterine operations, that had embryo reduction, and cases with missing or insufficient birth information were excluded from the study.

Variables such as age of mothers in both groups, gravidas, parities, level of education of mothers, time since the previous pregnancy, BMIs, weight gained during pregnancy, gestational week based on the last menstrual date and ultrasound, birth weights of infants, systolic and diastolic blood pressures, hemoglobin values, blood glucose levels, white blood cell counts, smoking history, follow-ups at the hospital, gender of babies, and seasonal distribution of stillbirths were evaluated. While assessing the data obtained from the study, SPSS for Windows 22.0 software was used for statistical analyses. While analyzing the study data, mean, median, and standard deviation that are descriptive statistical methods were utilized. Compatibility of study group to normal distribution was carried out by using Shapiro Wilks Normalization Test. As data was not compatible with normal distribution, Mann-Whitney U test was utilized for the comparison of parameters between the groups. Chi square test was used for the comparison of qualitative data. The results were evaluated in 95% confidence interval and significance was assessed at $p<0.05$ level.

Results

In our study, the stillbirth rate was found as 14 per million (/106) and stillbirth in risk-free population at 37-42 weeks was 1.05 per mill. Mean gestational week of the stillbirth group was 39.07 ± 1.34, and this was established as significantly high compared to live births (p: 0.005). Mean weight of stillborn babies in our study (3087gr) was established as statistically significantly low compared to babies in the control group (3311gr) ($p<0.05$). Antenatal polyclinic follow-up count in the stillbirth group was lower compared to the control group so as to manifest a statistical significance ($p<0.05$). BMI, hemoglobin levels, and systolic blood pressures, of mothers that had stillbirth were statistically significantly higher compared to the control group ($p<0.05$). Any statistically significant difference in mean maternal age, gravida and parity counts, level of education, weight gained during pregnancy, smoking history, and fetal gender distribution was not established between the groups in our study ($p>0.05$).

Table 1: Comparison of Data Based on the Groups (Mean±SD)

	Case (n=80)	Control (n=80)	P
Gestational Week	39.07 ± 1.34	38.51 ± 1.23	**0.005**
Age	28.37 ± 6.47	27.37 ± 5.78	0.38
Gravida	2.45 ± 1.69	2.23 ± 1.13	0.99
Parity	1.12 ± 1.39	0.96 ± 0.89	0.78
Time between pregnancies	3.50 ± 4.16	3.11 ± 3.49	0.96
BMI	30.90 ± 4.81	29.19 ± 4.14	**0.02**
Systolic Blood Pressure	112.87 ± 10.21	108.25 ± 8.96	**0.006**
Diastolic Blood Pressure	69.50 ± 8.55	68.75 ± 7.18	0.55
Infant Birth Weight	3087.50 ± 431.84	3311.87 ± 407.94	**0.000**
Blood Glucose	96.82 ± 34.27	88.04 ± 21.00	0.09
White Blood Cell	11636.87 ± 3060.18	15578.75 ± 23518.58	0.38
Hemoglobin	14.78 ± 16.86	11.72 ± 1.20	**0.007**
Weight Gained during Pregnancy	12.58 ± 4.28	11.15 ± 3.98	0.88

Data were given as mean ± Standard Deviation. P<0.05 was accepted as statistically significant. Mann-Whitney U Test.

Table 2. Distribution of Properties Between the Study Groups

	Groups	Case (n=80)	Control (n=80)	p
Gestational Week	37	12 (15)	17 (21.3)	**0.005**
	38	16 (20)	29 (36.3)	
	39	19 (23.8)	18 (22.5)	
	40	23 (28.8)	9 (11.3)	
	41	6 (8.8)	6 (7.5)	
	42	1 (3.8)	1 (1.3)	
BMI	18.5≤	0	0	**0.02**
	18.6-24.9	9 (11.3)	17 (21.3)	
	25-29.9	25 (31.3)	29 (36.3)	
	≥30	46 (57.5)	34 (42.5)	
Education Level	Illiterate	10 (12.5)	4 (5)	0.23
	Elementary School	27 (33.8)	26 (32.5)	
	Middle School	21 (26.3)	19 (23.8)	
	High School	13 (16.3)	23 (28.8)	
	Higher Education	9 (11.3)	8 (10)	
Follow-up Status	Yes	26	50	**0.000**
	No	54	30	
Smoking	Yes	3	6	0.49
	No	77	74	
Sex	Female	31	38	0,29
	Male	49	42	

Date were given as n (%). P<0.05 was accepted as statistically significant.

Discussion

Total number of births in our clinic in five years between 2011 and 2015 was 90,557 as obtained from the department of statistics in our hospital. Between these dates, stillbirths occurring at 28th week and above were 1,326. Of these cases, stillbirths that occurred in the group containing cases at 37-42 weeks bearing no risk factors, that were being followed up in antenatal polyclinic or that were referred to us by outside centres upon receiving diagnosis intrauterine fetal death were 96.

Global stillbirth rate is about 14 per million. Our stillbirth rate between the weeks of 37 and 42 in pregnancies without any risk factors was found as 1.05 per mill.

We established in our study that term stillbirths in the risk-free group may have a correlation with advanced gestational week, increased body-mass index, increased systolic blood pressure, and increased hemoglobin levels of the mother. In addition, we also established the need to count insufficient antenatal follow-up among the factors increasing the stillbirth risk.

Many studies show the advanced maternal age as an independent risk factor (10). Intrauterine fetal loss significantly increases especially above the maternal age of 35. Ling Huang et al. established that the stillbirth rate statistically significantly increases as the maternal age advances. An increase by 1.26-1.92 times was found in women aged 35 and above compared to women below the age of 35 (11) . In our study, a statistically significant difference was not established between the mean ages of case and control groups (p>0.05).

We believe the determining factor of this are our scans in certain week intervals and increased number of exclusion criteria. In addition, the fact that age distribution was not equal may be counted as one of the reasons.

Multiparity is also deemed a risk factor due to it impacting the physiology of the mother in stillbirth cases. In addition, nulliparity is an independent risk factor when analyzed on its own or together with other risk factors. In a study carried out by Adrienne Gordon et al., while a significant difference was not found between the multipara and nullipara, a significant risk increase was established in nulliparous women aged above 40 as subgroup. (12). In our study, too, a statistically significant difference was not found between the gravidas of case and control group members (p>0.05). There are studies in literature that reached conclusions similar to our study (13).

Socioeconomic status was believed to have had an impact on the number of follow-ups conducted at the hospital and the feeding level of the mother, and similarly, it was accepted as a preventable risk factor due to the effect of the education level of the mother on the awareness and its contribution to figure out the pregnancy risk factors at early stages and prevent them. According to literature, while low levels of education pose 1.5 times adjusted relative risk compared to high levels of education, medium levels of education were established as having 1.4 times adjusted relative risk (14). Due to the fact that, in our hospital, patients with low levels of income and low levels of education receive treatment, a difference was not observed between the groups in terms of the levels of education.

However, a statistically significant difference was established between the case and control groups in terms of follow-up conditions ($p<0.05$). Number of unfollowed cases in the case groups was found as higher compared to the control group, which manifests similar characteristics with the literature.

Without a doubt, one of the most discussed issues in recent years among stillbirth risk factors is BMI. According to the study by Addo et al., obese patients were observed to be shorter, older, and gained more weight during pregnancy, and as a result, the stillbirth rate was found significantly higher (OR=3.12 %95 Cl :1.42-7.57)(15). Whiteman et al. also established that stillbirth risk increases parallel to the rise in BMI (16). In our study, too, a statistically significant difference was found between BMIs of case and control group patients ($p<0.05$). Based on our study, rise in the BMI increased the stillbirth risk. In this study, a statistically significant difference was not established between the average weight gained during pregnancy in both groups ($p>0.05$). As a result, high BMI levels found in the case group suggests that the case group was pre-gestational overweight and that this finding show similarities in numerous studies in literature (16).

It was believed that male fetal gender possessed higher risk in terms of stillbirth, and the reason for this was suggested as the physiological differences between genders and their effects on the mother's physiology. It was also argued that the differences between male and female placenta could be a factor in this. There are contradicting publications on male gender and increased fetal death risk (17, 18). In our study, a statistically significant difference was not found between the groups in terms of fetal gender ($p>0.05$).

Studies appear to support that low birth weight increases stillbirth risk (19). In their study, Cnattingius et al. concluded that SGA (Small for Gestational Age) babies increased the stillbirth risk independently from other factors (20). In our study, too, birth weights of the case group were found as statistically significantly lower compared to the control group. It is important to take into consideration the low fetal weight determined during pregnancy follow-ups.

Smoking has been thoroughly investigated in terms of causing IUGR (Intrauterine Fetal Growth Restriction) and is among the agents whose effectiveness was manifested through dose-response curves (21). Smoking increases the stillbirth risk both on its own and indirectly thanks to other results caused by smoking.

(22). According to a meta-analysis in literature, smoking throughout the pregnancy increases stillbirth risk by 47% (23). Similarly, based on the data by the National Center for Health Statistics in the US, smoking increases relative stillbirth rate by 1.2-1.8 times (24). In our study, a statistically significant difference was not found between case and control groups in terms of smoking ($p>0.05$).

In literature, while anaemia is believed to have a correlations predominantly with preterm labour and SGA, there exist studies that show that hemoglobin levels above 13.9 are related with increased maternal morbidity and SGA (25, 26). In similar fashion, it was found that high hemoglobin levels measured at the first visit have correlations with negative pregnancy results and stillbirth risk (27). In our study, too, the hemoglobin levels of the case group were found as higher compared to the control group ($p<0.05$).

Seyom et al. found the stillbirth rate in patients with hypertension as 10.1% (28). In our study, too, systolic blood pressures of the case group were found as higher compared to the control group while complying with the literature.

Correlation between gestational week and stillbirth is still among the leading discussion topics. According to their study, Nicholson et al. established that there occurred an increase in stillbirth incidence in 7 years especially since the implementation of 39-week rule (29). They involuntarily commenced a discussion that the 39-week rule may be damaging. In our study, mean gestational week of the case group was 39.07 ± 1.34 and mean gestational week of the control group was 38.51 ± 1.23, and there was statistically significant difference ($p<0.05$). Accordingly, it should be borne in mind that gestational week poses a risk in the group with no risk factors in terms of stillbirth. It is necessary to be on alert bearing in mind stillbirths by assessing the patients in more detail in the coming weeks.

Conclusion

Consequently, we established in our study that term stillbirths in the risk-free group may be correlated with advanced gestational week, increased body-mass index, increased systolic blood pressure, and increased hemoglobin levels of the mother. In addition, we also showed that the insufficient antenatal follow-up should be counted among the factors increasing the stillbirth risk.

Results of this study may contribute to gravitating towards primary preventive healthcare programmes that specifically contain antenatal care. Stillbirth rates may be decreased by expanding the antenatal care services in primary and secondary centres, detecting high-risk pregnancies at earlies gestational stages, and taking necessary medical precautions.

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Cutaneous sporotrichosis with dermoscopic features: A case report

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Abstract

Objective: Sporotrichosis is a fungal skin infection caused by Sporothrix schenckii. We report a 70- year-old female presenting with an erythematous nodule on her right forefinger after a rose thorn prick.

Material and Methods: The nodule was histopathologically diagnosed as sporotrichosis. Dermoscopy of the nodule revealed structureless white areas with a lobular arrangement, prominent scaling, blood spots, and polymorphous vessels including coiled, punctate and looped vessels. Dermoscopy has opened a new horizon in the diagnosis of skin infections in recent years.

Conclusion: The dermoscopic features of sporotrichosis have not yet been reported as far as we are aware. We believe that coexistence of the above features, which could be thought to be nonspecific when seen separately, may be of diagnostic significance and a helpful tool in the diagnosis of cutaneous sporotrichosis.

Keywords: cutaneous sporotrichosis, Sporothrix schenckii, fungal, dermoscopy, histopathology

Introduction

Sporotrichosis is a fungal skin infection caused by the dimorphic fungus Sporothrix schenckii. The gold standard for the diagnosis is still the isolation and identification of the Sporothrix species. Dermoscopy is used as a diagnostic tool in many skin disorders and has opened a new horizon in the diagnosis of skin infections in recent years. The dermoscopic features of sporotrichosis have not previously been reported.

Case

A 70-year-old female presented with an erythematous, painless nodule on her right forefinger. The patient's history revealed that she had been pricked by a rose thorn while gardening and that the lesion had developed afterwards about 7 months ago.

The nodule had gradually enlarged during this period. Physical examination revealed an erythematous, scaly nodule measuring about 1x1 cm (Figure 1).

Mild pruritus was present. Dermoscopy showed numerous structureless white areas in a lobular arrangement, prominent scaling, blood spots, and polymorphous vessels including coiled, punctate and looped vessels (Figure 2).

Histopathological examination of the skin biopsy revealed the hyperkeratosis, parakeratosis, epidermal hyperplasia, neutrophilic microabscess formations, eosinophilic yeast-like organisms with surrounding of eosinophilic material (PAS+), mixed type inflammatory cells infiltrate the overlying keratin layer, epidermis and dermis (Figure 3).

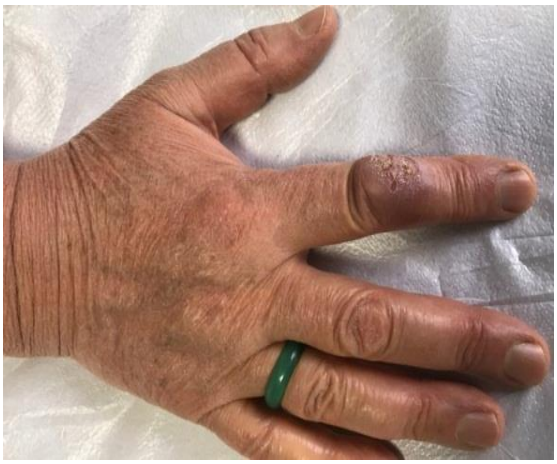

Figure 1: Erythematous nodule on the right forefinger

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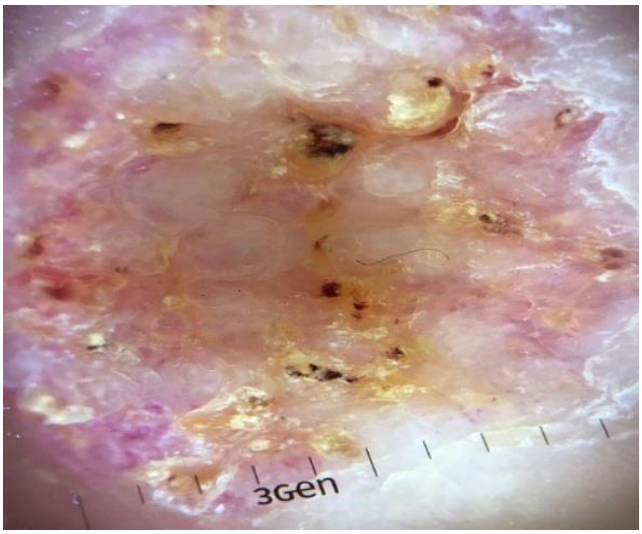
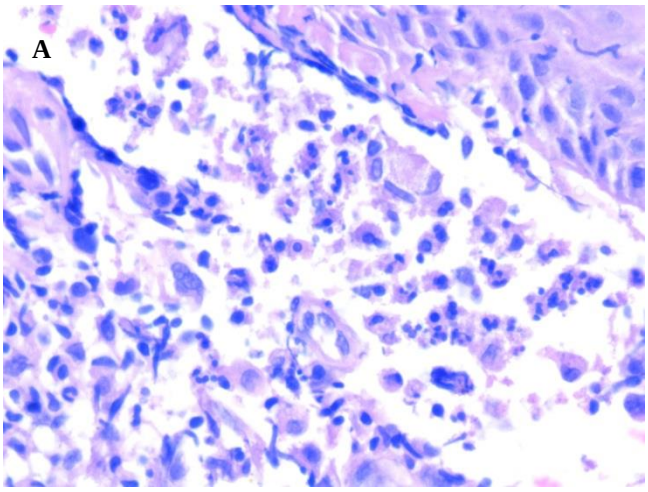


Figure 2: Numerous white structureless areas in lobular arrangement, prominent scale, blood spots, polymorphous vessels including coiled, dotted and looped vessels on dermoscopical examination



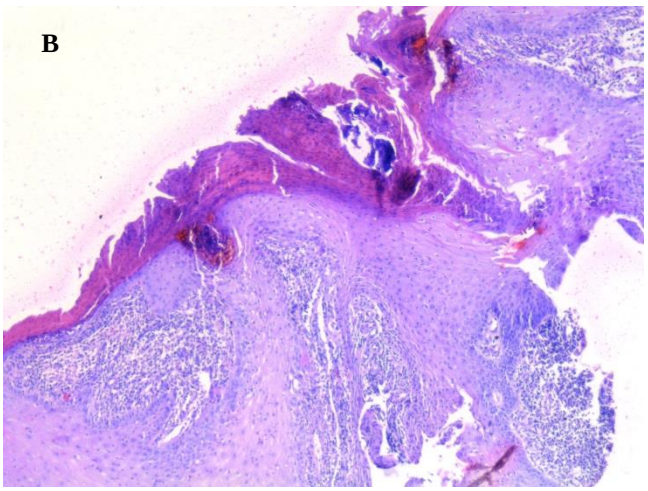


Figure 3A, 3B: Eosinophilic yeast-like organisms with surrounding of eosinophilic material; Hyperkeratosis, parakeratosis, epidermal hyperplasia, neutrophilic microabscess formations, mixed type inflammatory cells (H&E, x400; H&E, x100).

Discussion

Sporotrichosis (rose gardener's disease) caused by Sporothrix schenckii is the most common subcutaneous mycosis. The disease can present in the lymphocutaneous, fixed cutaneous or disseminated cutaneous form (1,2). The infection usually results from the inoculation of the agents on the skin or mucosa following trauma with contaminated plant materials, leading to an increased risk in farmers, gardeners, florists and foresters (2).

Lymphocutaneous sporotrichosis is the most frequent form and accounts for about 75% of the cases (2). Our diagnosis in this case was lymphocutaneous sporotrichosis as well. The initial lesion appears as a papulonodule in this form. The nodules can then ulcerate and produce a purulent discharge. Cutaneous sporotrichosis may also present as large ulcers with well-defined borders, and papulopustular, vegetative, infiltrative and crusty lesions (3). The differential diagnosis includes leishmaniasis, cat-scratch disease, cutaneous nocardiosis, chromomycosis, syphilis, granuloma annulare, and pyoderma gangrenosum.

The gold standard for the diagnosis of sporotrichosis is the isolation and the identification of the Sporothrix species from clinical samples. Histopathology, serology and molecular studies (PCR) are the other current diagnostic methods (4). Dermoscopy is a non-invasive diagnostic tool that is mainly used for evaluating pigmented skin lesions. It is now also widely used for the diagnosis of various inflammatory and non-inflammatory disorders of the skin.

Dermoscopy of skin infections and infestations, i.e., "entodermoscopy", has opened a new horizon in the diagnosis of these disorders as a fast and practical method in recent years. There is increasing evidence showing dermoscopy to be helpful in facilitating clinical recognition, confirming the diagnosis, making the differential diagnosis, and even monitoring the treatment response (5).

Specific dermoscopic criteria have been described for several infectious diseases including lupus vulgaris, leishmaniasis, scabies, pediculosis, HPV infections, molluscum contagiosum, and tick bites (6). The dermoscopic features of sporotrichosis have not previously been reported, due to literature search. We dermoscopicallydetected numerous structureless white areas in a lobular arrangement corresponding to hyperkeratosis and acanthosis of the epidermis with prominent scaling correlating with parakeratosis. A polymorphous vascular pattern, composed of two or more different vascular patterns, can be a dermoscopic sign for malignant skin lesions and especially melanoma (7). It can also be seen in benign skin lesions such as eccrine poroma and cutaneous leishmaniasis (8,9). We observed polymorphous vessels including coiled, punctate and looped vessels in our case, indicating that cutaneous sporotrichosis is another skin disease that can display a polymorphous vascular pattern. Observation of blood spots, as observed in our case, is another dermoscopic finding and can be associated with the itching and scratching.

Conclusion

Some dermoscopic features can be highly specific to the disease as in the example of scabies, which shows a "jet with contrail" appearance, while some dermoscopic signs can be seen in multiple disorders and are therefore considered 'nonspecific' (6). However, a nonspecific feature can also help in the diagnosis and differentiation of a disorder in the presence of other dermoscopic criteria. Although we cannot say any of the dermoscopic features we observed was particularly specific for cutaneous sporotrichosis, the lobular arrangement of structureless white areas, and the presence of prominent scaling, blood spots and a polymorphous vascular pattern indicated that sporotrichosis displays a distinct dermoscopic pattern, which needs to be confirmed on larger series. We believe that dermoscopy could become a helpful non-invasive tool for the diagnosis of cutaneous sporotrichosis.

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