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ORIGINAL ARTICLE

Evaluation of the effectiveness of treatment of vulvar lichen sclerosus et atrophicus. Analysis of own material and review of the literature.

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Abstract

MATERIAL AND METHODS: The study group included eleven female patients aged 18–77 years with a diagnosis of lichen sclerosus. Basic therapy consisted in the application of clobetasol in the first month and then once a day for the following two months. Then, clobetasol was recommended once a week until full resolution of the symptoms.

RESULTS: In nine patients with three-month basic therapy with clobetasol we observed a reduction of symptoms. Improvement of skin lesions was obtained in seven patients. After maintenance therapy lasting from four to twelve months the relapse of symptoms was observed in four women. Five women did not experience a relapse of the disease. The ointment with testosterone was applied in five women. Two women had poor tolerance of this drug. Two patients stopped the treatment after one month and after 11 months of using testosterone due to the relapse of the disease. One patient with good tolerance is currently continuing the therapy.

CONCLUSIONS: Vulvar lichen sclerosus et atrophicus is a chronic condition requiring long-term treatment. Topical use of steroids as first-line drugs bring a good local control of lesions in most women, yet further search of other possible causes of LSA is necessary.

INTRODUCTION

Lichen sclerosus et atrophicus (LSA) is a chronic condition affecting skin and mucous membranes. It was first described in 1887 (Fistarol & Itin, 2013). Although it can occur at any age, there are two peaks of incidence. The first one is in childhood at the average age of 7.6 years, while the second one is in the post-menopause period in women at an average age of 60 years. This disease is diagnosed six times more often in women than in men (Fistarol & Itin, 2013; Powell & Wojnarowska, 1999). Both manifestation and lesions are quite different in the course of this dermatosis. Nearly 85–98% of cases involve the anogenital region (Powell & Wojnarowska, 1999). Almost always the condition affects the labia minora and labia majora, accompanied by the involvement of the clitoris (70%), perineum (68%) and perianal region (32%) (Lorenz *et al.* 1998). The vagina is sporadically affected. Longiotti *et al.* described a 54-year old female patient with vulvar lichen

sclerosus involving mucosa of the posterior vaginal vault (Longinotti *et al.* 2005). The exact incidence of this disease is not known as there are no screening tests provided, no epidemiological data, and the course of this condition is asymptomatic in around 15–40% of patients (Wallace, 1971). Most recent data indicate that the incidence of LSA is one in 60 women (Goldstein *et al.* 2005) and up to one in 30 women in a population of elderly women (Leibovitz *et al.* 2000). The aetiology of lichen sclerosus is not yet fully known but experts suggest that there are multiple underlying causes (Olejek *et al.* 2009). The most common factors include autoimmunological causes, inflammations, genetic conditions and a low level of sex hormones (Higgins & Cruickshank, 2012; Chi *et al.* 2012). Circulating IgG antibodies against extracellular matrix are found in 74% of LSA women (Oyama *et al.* 2003). In 21–28% of patients other autoimmune diseases also occur (Meyrick *et al.* 1988; Cooper *et al.* 2004; Olek-Hrab *et al.* 2013). Infection with Borrelia Burgdorferi is confirmed in 47% of LSA women (Fistarol & Itin, 2013). A reduced level of 5 α-reductase may be an aetiological factor (Friedrich & Kalra, 1984). The use of oral contraception containing gestagen which has antiandrogenic properties probably disrupts androdependent growth of the vulva. According to Sherman *et al.*, up to 12% of patients have a positive family history and in many cases the disease affects first degree-relatives (Sherman *et al.* 2010). The average time from the occurrence of first symptoms to diagnosis is 69 months (Vieira-Baptista *et al.* 2015). Spontaneous remissions are rare (Fistarol, 2013), and the risk of developing squamous cell carcinoma with underlying LSA poses a significant problem – it accounts for 4–5% of cases (Rawal *et al.* 2005; Olejek *et al.* 2009). Advanced age and the proliferation of squamous cells constitute independent risk factors for the development of malignant process.

The aim of this paper was to evaluate the efficiency of treatment of lichen sclerosus et atrophicus of the anogenital region in women.

MATERIAL AND METHODS

The study group included female patients aged 18–77 years with a diagnosis of lichen sclerosus confirmed on the basis of tests of the skin collected from the vulva. The patients were treated at the Gynaecologic Oncology Outpatient Clinic at 1st Department and Clinic of Gynaecology and Obstetrics of the Medical University in Warsaw over the period of 2014–2017.

Material for histopathological tests was harvested from the skin of the vulva and then fixed in buffered 10% formalin. The tissues were embedded in paraffin blocks in a typical manner. The 4-micron thin slices obtained were stained with haematoxylin, eosin and mucicarmine.

A histopathologic diagnosis of lichen sclerosus et atrophicus was made based on the following characteristics: epidermis atrophy, oedema and the unified structure of the upper layer of the dermis, and a profuse inflammatory infiltrate in the middle layer of dermis.

Basic therapy consisted in the application of clobetasol twice a day in the first month and then once a day at night for the following two months. Then, clobetasol was recommended once a week until full resolution of the symptoms. After clobetasol was discontinued, maintenance therapy consisted of the application of ointments with vitamins (cod liver oil ointment, cholesterol ointment with vitamins), 1% cholesterol ointment with testosterone or creams with betamethasone. These ointments were used in monotherapy or were combined. After three months of treatment with clobetasol, daily use of emollients in the form of paraffin ointments was recommended as a constant element of therapy. Severe itching of the vulva and the occurrence of skin lesions were considered to be a relapse of the illness.

RESULTS

11 female patients aged 18–77 years were enrolled to the study (Table 1). Four patients had, in the past, undergone a hysterectomy with appendages, two of them due to endometrium adenocarcinoma and the other two due to benign lesions. Six patients had been treated for hypertension, one for type-2 diabetes, one for rheumatoid arthritis, one for hyperthyroidism, and one for hypothyroidism. Two of the women had had a hemicolectomy in the past due to colorectal cancer and intestinal volvulus.

In all of the women, skin lichen sclerosus et atrophicus was confirmed in the samples of tissue collected from the vulva. In three of them, mild vulvar dysplasia (VIN1) was also found. All the patients reported persistent itching of the vulva. In all eleven women, atrophic lesions involving the labia minora and labia majora were found, in eight of them (73%) the clitoris was affected, in four of them (36%) the perianal region.

In nine patients, a three-month basic therapy with clobetasol (Table 1) was instituted. All women observed a reduction of symptoms but no complete resolution of symptoms occurred in any patient. Improvement of skin lesions was obtained in seven (64%) patients. Maintenance therapy with clobetasol was instituted in the period from one to twelve months. The relapse of symptoms was observed in four women and in one patient, the relapse occurred as early as during maintenance therapy with clobetasol. In one patient, the disease reoccurred a month after the completion of the three-month basic therapy – this woman had not received maintenance therapy with clobetasol once a week. In two patients, the relapse occurred after 4 and 12 months following the completion of clobetasol treatment. Patients experiencing the recurrence of symptoms received a three-month clobetasol treatment once more, followed by therapy consisting of the application of this ointment once a week. Due to persisting symp-

Tab. 1.

	Age (yrs)	A	B	C (mths)	D (mths)	E (mths)	F (mths)	G (mths)	A	C (mths)	E (mths)	D (mths)	F (mths)
1.	66	yes	yes	8	1	3	–	4	yes	3	15	5	
2.	77	no	no	–	3	24	–	no			n/a		
3.	75	yes	yes	1	–	8	–	no			n/a		
4.	18	yes	yes	2	–	–	–	no			n/a		
5.	52	yes	yes	3	2	20	4	no			n/a		
6.	67	yes	no	7	11	12	–	12	yes	7 (3x/ week)	12	no	
7.	58	yes	no	6	–	–	–	0	yes	1	13	no	13
8.	58	yes	yes	4	–	24	–	no			n/a		
9.	70	yes	yes	–	–	–	–	1	yes	6	4	no	
10.	76	no	no	–	–	10	5	no			n/a		
11.	65	yes	yes	12	20	5	–	no			n/a		

A. Clobethasol administrated for three months – basic therapy B. Improvement of skin lesions C. Clobthasol administrated 1x/week D. Ointment with testosterone E. Ointment with vitamins F. Ointment with betamethasone G. Relapse of disease

toms during the maintenance therapy in one patient, it was recommended to use clobetasol three times per week and this resulted in clinical improvement. Such treatment was continued for seven months. The woman who experienced recurrence of the symptoms during treatment with clobetasol received another three-month treatment with this steroid followed by betamethasone therapy twice a week. She continues to receive this therapy until now due to the recurrence of symptoms when discontinuing the steroids. Five women did not experience a relapse of the disease. One18-year-old patient is currently undergoing maintenance therapy with clobetasol.

In two patients, the clobetasol treatment was not instituted since they had had surgical treatment for endometrium cancer in the past and supplementary radiation therapy within 12 months. One patient experienced a relapse of RA which required steroid therapy. These patients received therapy consisting of ointments with vitamins, testosterone and betamethasone, which resulted in a reduction in vulvar itching. Their skin lesions did not improve.

The ointment with testosterone was applied in five women. Two women had to discontinue this ointment after two and three months due to their poor tolerance of this drug. Two patients stopped the treatment after one month and after 11 months of using testosterone due to the relapse of the disease. One patient with good tolerance is currently continuing the therapy.

In three patients, treatment with betamethasone was instituted, which resulted in good topical control. In one patient, it was achieved after clobetasol therapy was completed. Due to mild itching of the vulva which periodically occurred, the ointment with testosterone was added to the therapy with vitamin ointment in maintenance therapy, however it was replaced with betamethasone due to poor tolerance. In the patient who had a history of endometrium cancer, the cream with betamethasone was added to the vitamin ointment due to persisting symptoms. In one patient, betamethasone was applied in the treatment when the condition reoccurred.

DISCUSSION

In our study, ZEA was detected in all the human milk samples, at a concentration ranging between 35.7 and 682 ng/L (median ZEA level, 173.8 ng/L). Detection of ZEA in human milk has been reported in two previous studies, which indicated that infants are exposed to this estrogenic contaminant and its main metabolites (Massart *et al.*, 2016; Rubert *et al.* 2014). In Massart *et al.*'s (2016) study, human milk samples were collected within the first six weeks after delivery from 47 healthy primiparous women in Naples, Italy; they were found to contain mean ZEA levels of 1.13 ± 0.34 μg/L (0.26–1.78 μg/L), which were correlated with the weight of the mother before pregnancy and at the time of delivery (Massart *et al.*, 2016). The ZEA levels reported in our study were lower than those reported in the previous Italian study (while the method of these two studies are different) : the median levels were 173 ng/L and maximum level was 682 ng/L in our study, while the mean level was 1130 ng/L in Italy (Massart *et al.*, 2016). A provisional maximum TDI of 0.5 μg/kg bw has been established for ZEA by the Joint Committee of the FAO/ World Health Organization (WHO) (Degen *et al.* 2017). In the study from Italy, the calculated TDI for ZEA was 0.2 μg/kg bw, which is slightly lower than the TDI of 0.5 μg/kg bw that has been set for adults. In our study, the TDI for ZEA was 0.03 μg/kg bw (range, 0.01–0.12 μg/kg), which is lower than the previously defined limit

as well as the values reported in the study from Italy (Massart *et al.* 2016; Rubert *et al.* 2014; Tonon *et al.* 2018; Degen *et al.* 2017). In an Italian study, no correlation was found between human milk ZEA levels and maternal dietary habits (Massart *et al.*, 2016). In the present study, however, human milk ZEA levels were associated with maternal consumption of meat, fish, dry fig, dried apricot, flaked red spice and spice, but the levels were lower than the threshold levels reported. It is possible that neonates are more susceptible than adults to the estrogenic effects of ZEA, as a result of higher internal exposures due to metabolic and physiological immaturity (Warth *et al.* 2016; Zinedine *et al.*, 2007; Kowalska *et al.* 2016; Degen *et al.* 2017). Degen *et al.* (2017) calculated exposure for a single dose or continuous daily intake of some mycotoxins, including ZEA, and reported that it can exceed the age-adjusted TDI values for infants; therefore, the safety of exclusively breastfed infants should be considered when setting the TDI.

In this study, human milk DON levels were found to vary between 400 and 14997 ng/L, with the median DON levels in infants who were exclusively breastfed being 3924 ng/L; further, the calculated median TDI for DON was 726 ng/kg bw (range, 74–2774 ng/kg). In this study, the calculated median TDI for breastfed newborns was 0.75 μg/kg bw (range, 0.24–2.77 μg/kg), which is lower than the previously set TDI of 1 μg/kg bw (Degen *et al.* 2017). However, in the present study, 36% of the breastfed infants had TDI >1 μg/kg bw. In contrast, Tonon *et al.* (2018) evaluated human milk DON levels (as well as the aflatoxin M1 and ochratoxin A levels) among 86 nursing mothers, but did not detect DON in any of the samples (even though the LC-MS/MS findings were indicative of DON in four samples). In addition, aflatoxin M1 and ochratoxin A were also not detected in the samples (Rubert *et al.* 2014). However, we previously found higher ochratoxin A levels in human milk samples in our study population than in other studies in Turkey (Warth *et al.* 2016; Dinleyici *et al.* 2018). The difference between our studies and other published ones might be related to geographical and diet-related exposure differences. A previous study that evaluated the urine DON levels among newborns reported that there was no significant difference in the mean urinary DON level across the different weaning categories; however, the authors concluded that most of the children with detectable levels of DON were either partially breastfed or fully weaned (EFSA J 2013). Chronic administration of DON in animals causes weight loss, anorexia, decreased nutritional efficiency, immune disorders such as immunosuppression and immunostimulation (depending of the dosage and exposure frequency), and increased susceptibility to facultative pathogens (Raiola *et al.* 2015; Sobrova *et al.* 2010). DON was found to be a frequent contaminant in a large number of samples of cereal grains such as wheat, maize, oats, barley, rye, and rice, and in some processed food products such as wheat flour, bread, breakfast cereals, noodles, baby and infant foods and cooked pancakes (EFSA J 2013).

In this study, human milk DON levels were positively correlated with maternal meat consumption. Mycotoxins can appear in the food chain because crops might be consumed by livestock. According to the metabolism of the individual, the ingested mycotoxins could accumulate in different organs or tissues, and eventually enter the food chain through meat, milk or eggs (Alshannaq and Yu, 2017; Marin *et al.* 2013; Raiola *et al.* 2015; Richard 2007). Thus, the DON contamination levels in meat products are far lower than those in cereal-based food and feeds, but they may still be cause for concern. In Turkey, DON levels have been evaluated in dairy cattle, beef cattle, and lamb–calf feeds with ELISA, and the DON levels in feed samples were found to be lower than the legal limits in Turkey (Kocasari *et al.* 2013). However, in this study, we did not analyze food consumed by the mothers including meat; therefore, it is difficult to directly correlate meat consumption with human milk DON levels based on the present findings. Further studies should be conducted in the future to understand this correlation better.

DON is probably the best known and most common contaminant in food and feed; it is found in more than 90% of food samples and is a potential marker for other mycotoxins. DON may also coexist with ZEA, which is produced by fungi of the same genus. In support of this, human milk DON levels were positively correlated with human milk ZEA levels in our study, despite the lower ZEA levels and higher DON levels observed.

We employed enzyme-linked immunosorbent assay (ELISA) for analysis, as it is the most commonly used assay for mycotoxin determination. Thin-layer chromatography is another effective method for assessing DON. ELISA provides rapid screening, and many kits are commercially available for the detection and quantification of all major mycotoxins including ZEA and DON. This technique provides is rapid, specific, and relatively easy to use for the analysis of mycotoxins in food. However, ELISA has certain disadvantages including potential cross-reactivity and dependence on a specific matrix (Alshannaq and Yu, 2017).

In conclusion, the presence and higher levels of measurable ZEA and DON in nursing Turkish mothers indicate that they are exposed to mycotoxins as a result of their dietary habits. Very little is known about the effects of ZEA and DON on childhood growth and development, but children are believed to be particularly vulnerable, since their rate of food consumption is higher than that in adults. Such data will improve risk communication and will be informative to policy makers. Such research activities will also help to provide support to and protect those who are more susceptible to the negative effects of mycotoxins and other contaminants. These toxins have long-term side effects; however, it is not recommended that mothers stop

breastfeeding their infants, as the advantages of breastfeeding are clear. Instead, comprehensive programs should be developed to regularly investigate and control these toxins in both human and animal food chains so that the amount of toxins can be reduced and their side effects can be prevented. Further regulations/recommendations are needed with regard to the dietary habits of nursing mothers so as to reduce the risk of maternal exposure to mycotoxins. More studies are also needed to provide new dietary recommendations for women during the pregnancy and lactation period.

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