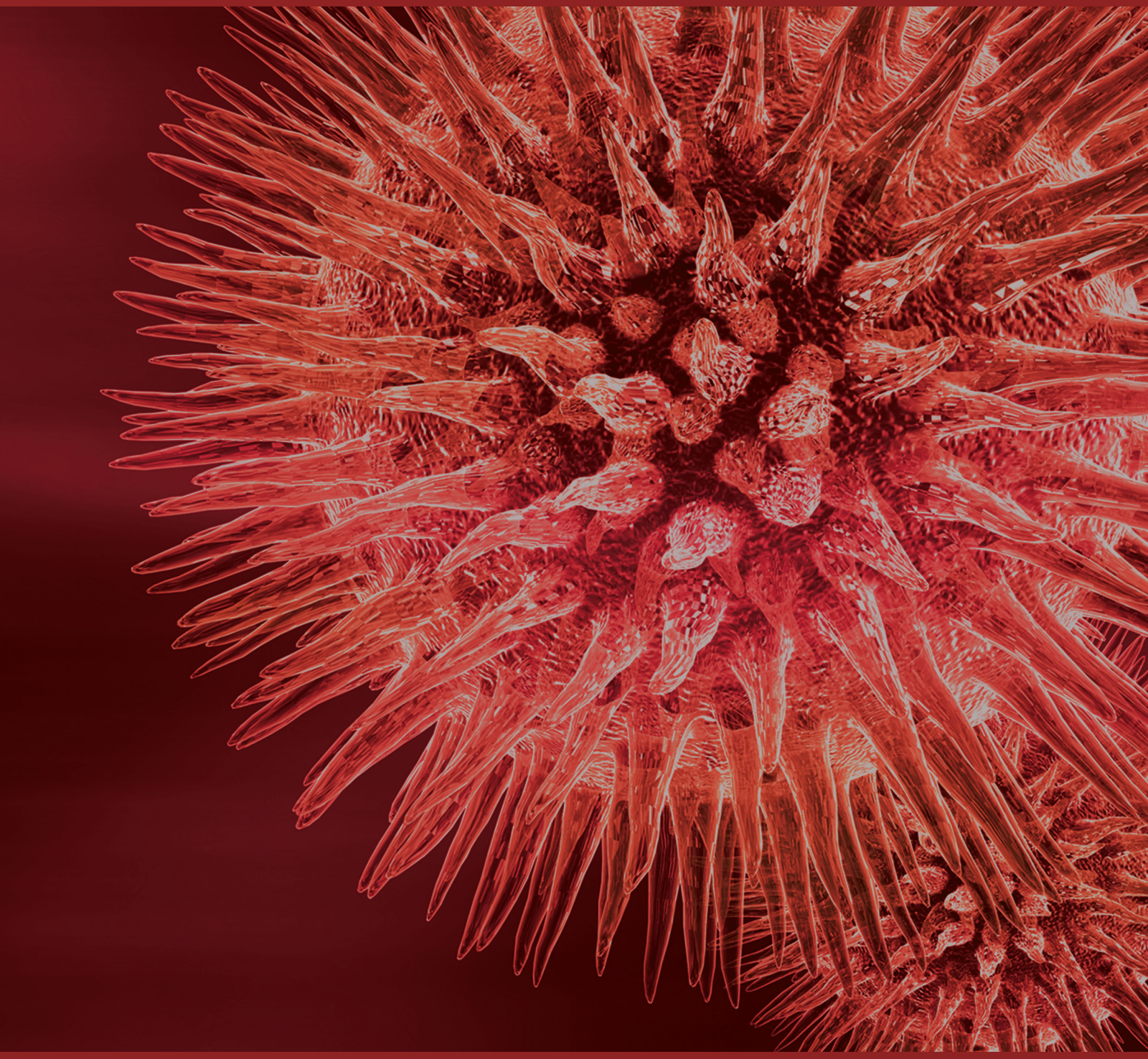
BioMed Research International

Women's Skin throughout Lifetime

Guest Editors: Gérald E. Piérard, Corinne Charlier, Philippe Delvenne, Philippe Humbert, and Claudine Piérard-Franchimont



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Editorial

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Gérald E. Piérard,[1] Corinne Charlier,[2] Philippe Delvenne,[3] Philippe Humbert,[4,5] and Claudine Piérard-Franchimont[6,7]

[1] *Laboratory of Skin Bioengineering and Imaging (LABIC), Department of Clinical Sciences, Liège University, 4000 Liège, Belgium*
[2] *Department of Clinical Toxicology, University Hospital of Liège, 4000 Liège, Belgium*
[3] *Department of Pathology, Unilab Lg, University Hospital of Liège, 4000 Liège, Belgium*
[4] *Department of Dermatology, University Hospital Saint-Jacques, 25030 Besançon, France*
[5] *Inserm U645 Research Unit IFR133, 25030 Besançon, France*
[6] *Department of Dermatopathology, Unilab Lg, University Hospital of Liège, 4000 Liège, Belgium*
[7] *Department of Dermatology, Regional Hospital of Huy, 4500 Huy, Belgium*

Correspondence should be addressed to Gérald E. Piérard; gerald.pierard@ulg.ac.be

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The distinction between skin of men and women stems as much from biologic differences as from their different social roles and status in various societies. Currently, there is agreement among clinicians and researcher scientists to distinguish between different episodes in female life according to their hormonal status. They influence the skin physiology and the risk of diseases and possibly alter the quality of life. The present special issue contains ten papers focused on recent developments in the field of women's skin and mucosae. Two papers deal with physiological aspects of breasts prior to surgical intervention, as well as the skin response to seasonal environmental changes. One paper is focused on new developments in toxicological aspects related to selected hormone disruptors. Seven other papers focus on peculiar aspects of a series of skin disorders developed in women.

The paper entitled "*A methodological evaluation of volumetric measurement techniques including three-dimensional imaging in breast surgery,*" by H. Hoeffelin et al., describes objective and noninvasive methods for assessing breast volume. The 3D LifeViz system (Quantificare) was used in various settings (in situ on corpse dissection, on control prostheses, and in clinical conditions). Such a system was compared to other methods (CT scanning and Archimedes' principle) under the same conditions. The parameters of feasibility, safety, portability, minimal patient stress, and limitations (underestimation of the in situ volume, subjectivity of contouring, and patient selection) of the LifeViz 3D system indicate similar benefits compared to other measurement methods. The prospects of this method appear promising for a series of applications in clinical practice in order to limit the subjectivity of breast surgery.

The paper entitled "*The weather-beaten dorsal hand clinical rating, shadow casting optical profilometry, and skin capacitance mapping,*" by M. Delvenne et al., is an original work about a common condition linked to seasonal skin presentation associated with environmental changes. The withered skin surface changes were assessed during the four seasons. Among 47 menopausal women completing the study, 31 volunteers were on hormone replacement therapy (HRT) and 16 did not use HRT. Skin xerosis and scaliness were rated following clinical assessments. In addition, skin withering of the dorsal hands was assessed by computerized shadow casting optical profilometry. Skin capacitance mapping was also performed. Marked changes over the seasons were recorded corresponding to the combination of patchy heterogeneous stratum corneum hydration and heterogeneous skin surface roughness. These features likely resulted from variations in the environmental temperature

and moisture. Daily stress due to alternate outdoor and indoor conditions was possibly involved in the skin condition. The severity of changes revealed by clinical inspection was not supported by similar directions of fluctuations in the instrumental assessments. Such contradiction was in fact due to different levels of scale observation. The clinical centimetric scale and the instrumental inframillimetric scale possibly provided distinct aspects of the biological impact.

The paper entitled "*Measurement of urinary biomarkers of parabens, phthalates, and benzophenone-3 in a Belgian population*," by L. Dewalque et al., addresses the concerns about hormone disruptors. Phthalates, parabens, and benzophenone-3 (BP3) are commonly present in plasticizers, antimicrobial conservatives, and UV-filters, respectively. They exhibit endocrine disrupting properties yielding, for instance, to skin malignant melanoma. Humans are exposed to such chemicals through different sources like food, personal care preparations, and cosmetics. In this study, the exposure to five phthalates, four parabens, and BP3 was assessed by measuring urinary levels of their biomarkers in samples collected from 261 volunteers living in the Liège region of Belgium. The analyses were carried out by liquid chromatography tandem mass spectrometry (LC-MS/MS) with deuterated standards. The phthalate metabolites, BP3, and most of the parabens were detected in 82.8 to 100.0% of the samples. For most of these chemicals, the exposure patterns seemed to differ between children and adults. In addition, differences were perceived between males and females, especially with significantly higher concentrations of parabens in females. If a correlation was established between the incidence of some cutaneous pathologies, for example, malignant melanoma, and the presence of endocrine disrupting chemicals in biological samples, the present results are puzzling. Further studies on the subject are necessary to clarify this relationship.

In the paper entitled "*Pruritus in female patients*," JMRG Lambert reviews the global understanding about pruritus affecting some women. Pruritus is a frequent symptom in a number of skin diseases. This review was focused on specific itch problems specific to women, namely, pruritic vulvar dermatoses, and specific pruritic dermatoses of pregnancy. The characteristics of the vulva and the hormonal changes during the different age periods make these dermatoses very particular. It seems that vulvar diseases remain underdiagnosed and undertreated. Pruritic vulvar diseases have a severe impact on quality of life. The most common pruritic diseases concern atopic and contact dermatitis, psoriasis, lichen sclerosus, lichen planus, and infectious vulvovaginitis. The diagnostic issues of these diseases are discussed and the general principles of therapy are covered.

In the paper entitled "*The female pattern hair loss in 2013: Review of etiopathogenesis and diagnosis,*" A. Vujovic and V. Del Marmol review concepts regarding female pattern hair loss (FPHL), representing the most common hair loss disorder in women. Initial signs possibly develop in teenagers leading to a progressive hair loss with a characteristic distribution pattern. The condition is characterized by progressive replacement of terminal hair follicles over the frontal and vertex regions by miniaturized follicles. This evolution leads progressively to a perceptible reduction in hair density. Women diagnosed with FPHL may undergo significant impairment of quality of life. FPHL diagnosis is mostly clinical. Depending on patient history and clinical evaluation, further diagnostic testing is occasionally useful.

In the paper entitled "*Female gender and acne disease are jointly and independently associated with the risk of major depression and suicide: a national population-based study,*" Y. C. Yang et al. address the association of acne, poor self-esteem, and social phobia in Taiwanese people. Previous studies based on questionnaires from several thousand adolescents had showed that acne was particularly associated with major depression and suicide. Using a 2006 database from the National Health Insurance in Taiwan, patients with acne, major depression, and suicide were considered. A total of 51689 patients with acne were identified (17974 males and 33715 females) from 1 million subjects. The youths (7–12 years) had the highest prevalence of acne (15.87%). Major depression was more common in patients with acne (0.76%) than in controls (0.56%, $P < 0.0001$) regardless of gender. Multiple logistic regressions showed an increased risk to major depression in women without acne. The risk was further increased in women with acne. Similar increased risk of suicide was noticed in acneic women. In conclusion, acne and gender, independently and jointly, are associated with major depression and suicide. Special medical support should be warranted in females with acne for the risk of major depression and suicide.

The paper entitled "*Psoriasis: female skin changes in various hormonal stages throughout life—puberty, pregnancy, and menopause,*" by R. Ceovic et al., discusses the fate of psoriasis in women. Stress greatly affects both the hormone and immune systems. The severity of psoriasis often fluctuates or is influenced with each hormonal phase in women and this relationship affects some disease frequencies peaking at puberty, during postpartum, and menopause, while symptoms improve during pregnancy.

The paper entitled "*Vulvar skin disorders throughout lifetime: main dermatoses,*" by J. Doyen et al., addresses four representative vulvar dermatoses. Lichen simplex chronicus is a pathological condition related to friction. Such detrimental effects in the presence of other dermatoses have to be considered when therapeutic responses are unsatisfactory. Lichen sclerosus is a common vulvar dermatosis particularly in the elderly. Lichen planus is a distinct entity. Paget's disease, although a rare condition, represents a clinical and therapeutic challenge.

The paper entitled "*A clinical and pathological overview of vulvar condyloma acuminatum, intraepithelial lesions, and squamous cell carcinoma,*" by B. Léonard et al., is focused on some vulvar epithelial neoplasms. Condyloma acuminatum, intraepithelial neoplasia, and squamous cell carcinoma are three common vulvar lesions. Condyloma acuminatum is induced by low risk genotypes of human papillomavirus (HPV). Vulvar intraepithelial neoplasia (VIN) and squamous cell carcinoma have different etiopathogenic pathways and are related or not with high risk HPV types. This article reviews the main pathological and clinical features of these

lesions. A special attention is paid to epidemiology, pathological classification, and clinical implications of these diseases.

The paper entitled "*Streamlining cutaneous melanomas in young women of the Belgian Mosan region*," by T. Hermanns-Lê and S. Piérard, is a review article focused on sporadic cutaneous melanomas (SCM) developing in women during their childbearing age. This neoplasm has shown an increased incidence over the past few decades. The vast majority of these SCM were of the superficial type without any obvious relationship with a large number of melanocytic nevi. Signs of frequent and intense sunlight exposure were not disclosed by the extent in the mosaic subclinical melanoderma. A series of investigations point to a possible relationship linking the development of some of these SCM with the women hormonal status including the effect of hormonal disruptors. These aspects remain, however, unsettled and controversial. It is possible to differentiate and clearly quantify the SCM shape, size, scalloped border, and variegated pigmentation using computerized morphometry as well as fractal and multifractal methods.

We hope this special issue will not only help to understand and diagnose some skin disorders typically found in women, but also reinforce the development of better management modalities for these women skin disorders.

Gérald E. Piérard
Corinne Charlier
Philippe Delvenne
Philippe Humbert
Claudine Piérard-Franchimont

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Review Article

The Female Pattern Hair Loss: Review of Etiopathogenesis and Diagnosis

Anja Vujovic and Véronique Del Marmol

Department of Dermatology, Hôpital Erasme, Université Libre de Bruxelles, Route de Lennik 808, Anderlecht, 1070 Brussels, Belgium

Correspondence should be addressed to Anja Vujovic; vujovic.anja@yahoo.com and Véronique Del Marmol; v.marmol@skynet.be

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Female pattern hair loss (FPHL) is the most common hair loss disorder in women. Initial signs may develop during teenage years leading to a progressive hair loss with a characteristic pattern distribution. The condition is characterized by progressive replacement of terminal hair follicles over the frontal and vertex regions by miniaturized follicles, that leads progressively to a visible reduction in hair density. Women diagnosed with FPHL may undergo significant impairment of quality of life. FPHL diagnosis is mostly clinical. Depending on patient history and clinical evaluation, further diagnostic testing may be useful. The purpose of the paper is to review the current knowledge about epidemiology, pathogenesis, clinical manifestations, and diagnosis of FPHL.

1. Definition of FPHL

The FPHL is a *nonscarring* progressive thinning of hair. It results from a progressive decrease in the ratio of terminal hairs to shorter, thinner vellus hairs, a process known as follicular miniaturization [1]. This miniaturization follows usually a pattern distribution. In women, FPHL typically presents as a diffuse reduction in hair density over the frontal and vertex areas, but parietal and occipital regions may be involved [2].

2. Terminology

In the past, the term "androgenetic alopecia" (AGA) was the primary term used to refer to this condition in both men and women. The term "andro" from ancient Greek refers to male subjects and "genetic" referred to the contribution of heredity. Over the years, "female pattern hair loss" became the preferred term for this form of hair loss. This terminology helps to distinguish the different features of the condition in women versus men and shows the lack of clear hormonal contribution in many cases. Further, some authors use the terms "androgen-dependent FPHL" and "androgen-independent FPHL" to separate women who have FPHL due to androgen excess from those with normal androgen levels [3].

3. Epidemiology

FPHL is very common and increases with age in the Caucasian womenpopulations. In 2001, Norwood established the prevalence of FPHL at 19 percent in a series of approximately 1000 Caucasian women [4]. Although FPHL can occur at any time of life, the condition occurs the most following menopause. This age-related rise was clearly established in Norwood's series; FPHL was only detected in 4 of 121 women between the ages of 20 and 29 (3 percent), but in 41 of 140 women between the ages of 70 and 89 (29 percent) [4]. In a British study of 377 women, 38 percent of women over the age of 70 years had FPHL [5]. The prevalence of the condition seems to be lower in the Asian population [6, 7]. A Korean study shows that the prevalence of FPHL in Korean women at all ages was only 5.6%. Like in Caucasian women, this prevalence increases steadily with advancing age [7]. There

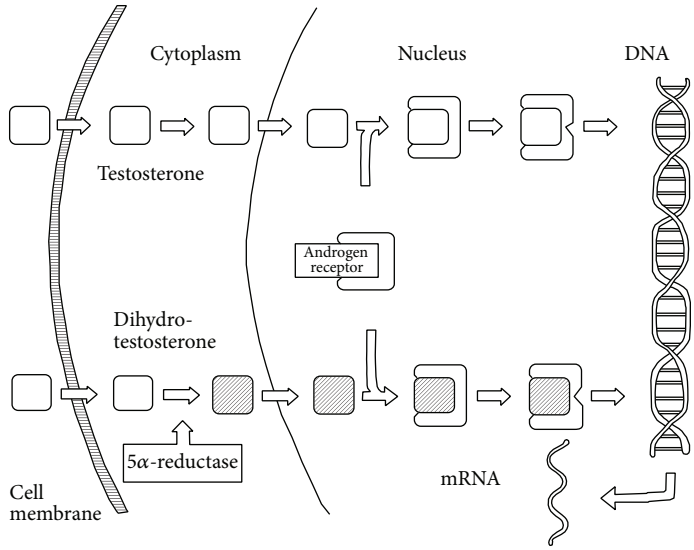

Figure 1: Schematic of the general mechanism of androgens action. Inside the cell, testosterone and DHT bind to the androgen receptor. Once the hormone has bound, the complex will bind to the DNA, altering the expression of specific androgens-dependent genes [10]. Reproduced by Thierry Huart.

are no published data over the prevalence of FPHL in African women.

4. Etiology and Pathogenesis

The visible thinning of hair of the scalp in FPHL results from a progressive decrease in the ratio of terminal hairs to shorter vellus hairs, a process called follicular miniaturization [8]. The mechanism through which this follicular transformation occurs in FPHL is not completely understood. Although the roles of androgens and genetic susceptibility in male AGA are well accepted, the degree to which these factors contribute to FPHL is less clear.

4.1. Androgens. The androgens role in common male baldness was first suspected by Hamilton in 1942. He notes that AGA does not occur in men who have never entered puberty, that baldness stops its progression in castrated men, and that testosterone replacements stimulate progressive balding [9]. In recent studies, AGA is described as a consequence of the direct effects of dihydrotestosterone (DHT) on the dermal papilla of susceptible hair follicles [10]. DHT is a more potent androgen, coming from the metabolism of testosterone by the action of the 5α-reductase. DHT binds to androgen receptors in hair follicles, more strongly even than testosterone, resulting in upregulation of genes responsible for the gradual transformation of terminal hair follicles to miniaturized hair follicles (Figure 1) [11]. These miniaturized hairs of various lengths and diameters are the hallmark of FPHL and AGA [10, 12, 13]. However, the number of follicles per unit area remains the same [14]. The pattern of hair loss, which typically spares the occipital scalp, reflects regional differences in the sensitivity of scalp follicles to androgens. Some authors have theorized that a similar process contributes to the development of FPHL, a concept supported by the observation that women with hyperandrogenism may develop early-onset FPHL [2]. However, most women with FPHL have no other signs or symptoms of hyperandrogenism and have normal androgen levels, indicating that our understanding of the pathogenesis of the disorder remains incomplete. The age-related increase in FPHL and the highest rates in postmenopausal women may suggest a protective role of the estrogen. Supporting this theory, Sawaya and Price conducted a study in 12 young women and 12 young men (ages from 14 to 33) suffering from AGA or FPHL [15]. Scalp biopsies were taken and androgens, expression of androgen receptor, type I and type II 5α-reductase, and cytochrome p-450 aromatase enzyme genes were measured in hair follicles. Both young women and young men had higher levels of type I and type II 5α-reductase and androgen receptors in frontal hair follicles compared to occipital hair follicles explaining probably the patterned hair loss. However, the levels in women were approximately half the levels in men [15]. The findings of this study suggest that the milder expression of FPHL may in part be the result of lower levels of 5α-reductase and androgen receptors in frontal follicles of women compared to levels in men. Additionally, young women had much higher levels of cytochrome p-450 aromatase, enzyme capable of converting testosterone to estradiol, in frontal and occipital follicles than men. Those notable increased aromatase levels seem to play a protective role in the development of hair loss in women [15]. Furthermore, supporting the androgen-dependent etiopathogenesis, low levels of sex hormone-binding protein (SHBG), glycoprotein that binds to androgens, inhibiting thereby their activities, have been linked to diffuse hair loss [16]. Another part of FPHL and AGA pathogenesis is the gradual shortening of the growth phase of hair follicles. Over the successive hair cycles, the duration of anagen phase shortens from a normal duration of a few years to only weeks to months [2].

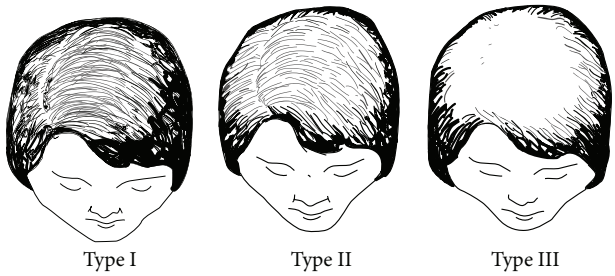

Figure 2: Ludwig pattern of hair loss in women. Three-point scale. Diffuse thinning of the crown region with preservation of the frontal hairline. Drawing by Thierry Huart based on Ludwig et al. [24, 25].

4.2. Genetics. There are few studies evaluating the genetic basis and inheritance pattern of FPHL [8]. One of them shows an incidence of 54% pattern hair loss in first-degree male relatives of age >30 years and 21% in first-degree female relatives >30 years. Those reports of the occurrence of both FPHL and AGA in individual families suggest that FPHL and AGA share a common genetic background [17, 18]. The two major susceptibility loci for the AGA in men are the androgen receptor (AR)/ectodysplasin A2 receptor (EDA2R) locus on the X-chromosome and a locus on chromosome 20p11, for which no candidate gene has yet been identified [19, 20]. Very recent studies show no involvement of the well-established locus on chromosome 20p11 in FPHL but suggested that the X-chromosomal locus containing the androgen receptor (*AR*) and the ectodysplasin A2 receptor (*EDA2R*) genes may be specifically involved in the pathogenesis of early-onset FPHL [21]. Moreover, an Australian genomewide association study suggested that the aromatase gene (CYP19A1) may contribute to FPHL [22].

5. Clinical Features

FPHL may have three different patterns [23]:

(1) diffuse thinning of the crown region with preservation of the frontal hairline: two scales are used to describe this pattern: the commonly 3-point Ludwig scale [12, 24] (Figure 2) and the 5-point Sinclair scale [25] (Figure 3);

(2) thinning and widening of the central part of the scalp with breach of frontal hairline, described by Olsen scale: Christmas tree pattern (Figure 4) [26];

(3) thinning associated with bitemporal recession; Hamilton-Norwood scale [27].

All these common patterns spare the occipital area, a phenomenon explained probably by hormonal influences explained above. This behavior's difference between the frontal/parietal follicles and the occipital follicles is found in other hair disorders like alopecia areata, a condition where occipital follicles affected by the ophiasis pattern are typically more resistant to regrowth [28]. These differences may result from the embryological derivation of the dermis in the two regions. It is known from avian embryology that the dermis of the frontal/parietal scalp is of neural crest origin, whereas the dermis of the occipital scalp is of mesodermal origin [29].

6. Associated Disorders

6.1. Psychosocial Dysfunction. Many women suffering from FPHL experience negative psychosocial effects related to the condition. In a questionnaire-based study, 70 percent of affected women reported that they were very to extremely upset about their hair loss. They experienced more feeling of negative image body and poorer self-esteem and had a less quality of life than the control group [30]. In another study, 88 percent of females with FPHL felt that the hair loss negatively influenced daily life [31]. Clinicians managing patients with FPHL should remain aware that the patient's perception of the severity of hair loss may be different from the clinical assessment of the severity and the psychosocial impact of the condition. In a recent questionnaire-based study of 104 women suffering from different hair loss disorders like alopecia areata, FPHL, or telogen effluvium, the patient's perception of the severity of hair loss was greater than the severity ratings given by the dermatologist. In addition, the personal rating of hair loss will be more closely correlated with the effects of hair loss on quality of life [32]. This psychosocial dysfunction related to FPHL is also experienced by adolescent girls suffering from the condition. Those teenage girls may experience poor self-esteem and impaired functioning at home, school, or work and in personal relationships [33].

6.2. Medical Conditions. Links between early-onset FPHL and insulin resistance and hypertension and increased cardiovascular risk have been described [34, 35]. Those conditions seem to be due to higher aldosterone, C-protein, D-dimers, and insulin levels in women suffering from FPHL than in control subjects. Authors recommend the determination of metabolic syndrome and ultrasound study of the carotid arteries to detect risk of developing cardiovascular disease in female patients with early-onset FPHL [35]. Recently, vertex pattern AGA in male patients of all ages was associated with an increased risk of prostate cancer [36]. There is no evidence in the current knowledge of an association between FPHL and the risk of any kind of cancer.

7. Clinical Diagnosis

7.1. General History. The physician should record age of onset and duration and progression of hair loss. The patients often describe a chronic hair loss with some increased periods of activity, particularly during autumn and winter. The patient should be asked about thinning and shedding. For thinning, most patients described an accentuation of the frontal, parietal, or vertex region, but a diffuse thinning is possible as well. The family history is more often positive, but a negative family history does not exclude the diagnosis. The patient should be asked about other familial hair disorders like alopecia areata or hirsutism, which may influence the further investigations. It is also important to exclude other

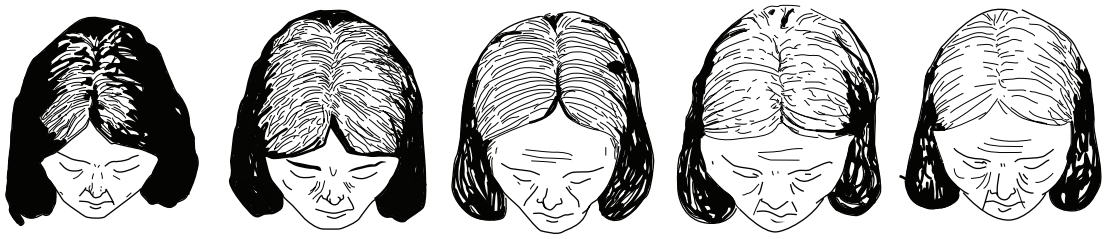

Figure 3: Sinclair scale: 5-point scale for grading of FPHL with diffuse thinning of the crown region with preservation of the frontal hairline. Drawing by Thierry Huart based on the Sinclair Scale, Sinclair et al. [25].

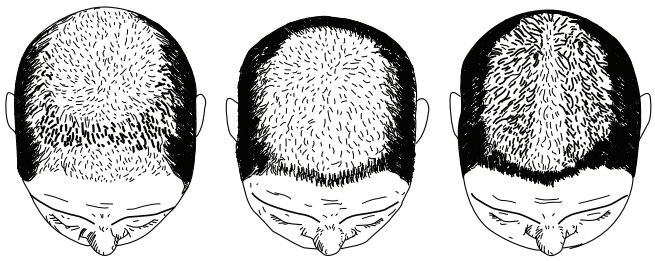

Figure 4: Olsen scale: Christmas tree pattern in female pattern hair loss. Thinning associated with bitemporal recession. Drawing by Thierry Huart based on Olsen scale, Olsen [26].

causes of hair loss whose untreated presence could affect the efficacy of the FPHL treatment. The presence of other medical disorders and newly diagnosed diseases within one year prior to first signs of hair loss and the medical treatment should be investigated. Other causes of hair loss such as diffuse effluvium due to iron deficiency, infection, thyroid dysfunction, or chronic deficient diet should be excluded. Some drugs such as chemotherapeutic agents, proandrogenic hormones, or antithyroids may cause diffuse hair loss. Moreover, some cosmetic habits (traction) or environmental factors like smoking [37] or UV-exposition [38] may induce increased hair loss in women. The experts all agree to ask about eating behavior, as chronic deficient diet or rapid important weight loss can trigger diffuse effluvium [23].

7.2. Gynecological History. A complete gynecological and obstetrical interrogatory that includes menarche, menstrual cycle (regular/irregular), menopause, amenorrhea, use of oral or systemic hormonal contraception, hormone replacement therapy, fertility treatment, problems in getting pregnant, gynecological surgery, pregnancies, births, miscarriages, and signs of hyperandrogenism (excessive body hair growth, acne, ect.) should be done to exclude influencing hormonal deregulations (e.g., hormone sensitive tumor) [39]. Impaired fertility, amenorrhea, irregular menstrual cycle, and signs of hyperandrogenism and hyperseborrhea may be indicative for polycystic ovary syndrome [40].

7.3. Physical Examination. A full skin examination that includes body and nails is advised in women complaining of hair loss. Nails abnormalities identification is not typical in FPHL but may differentiate the condition from other cases of hair loss like alopecia areata, iron deficiencies, or lichen planus [23]. A whole body examination should be performed to find other signs of possibly associated hyperandrogenism.

7.4. Scalp and Hair Scalp Examination. Scalp examination should focus on identifying the distribution of hair loss and the caliber of hairs in commonly involved areas. Findings consistent with FPHL include the detection of terminal hair loss, variation of hair caliber, and miniaturized hairs. Pull test identifying increased shedding of telogen hairs is typically negative except in active phases of FPHL. The most frequently used scales are the Ludwig (Figure 2) and the Olsen (Figure 4) scales, described previously. FPHL is a nonscarring alopecia, explaining that the scalp skin appears normal, but other clinical features such as inflammation, scarring, or hyperseborrhea can be associated and potentially aggravating the FPHL [41]. The physician should also consider scarring alopecia mimicking FPHL like frontal fibrosing alopecia [42].

7.5. Trichoscopy. Trichoscopy or scalp dermatoscopy is a noninvasive diagnostic tool, very useful for the diagnosis and followup of hair and scalp disorders [43]. Trichoscopy of FPHL is characterized by hair diameter variability greater than 20% [44] (Figure 5). Hair shaft variability can also be present in alopecia areata. However, in this pathology dermatoscopy shows uniform miniaturization instead of hair shafts with different degree of thinning. This hair diameter variability is very useful to detect early FPHL in children or teenage girls [45]. In 2009, Rakowska et al. [46] proposed major and minor dermoscopic criteria for the diagnosis of

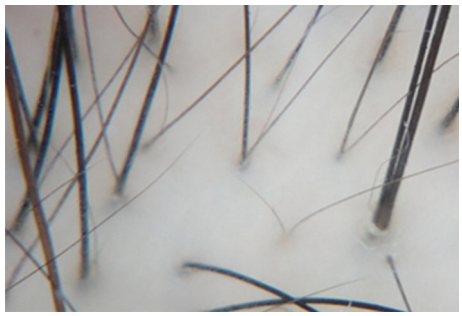

Figure 5: Trichoscopy of the frontal scalp in a female patient complaining of chronic hair loss. Trichoscopy shows FPHL: hair shaft variability greater than 10%, vellus hairs, and perifollicular discoloration.

FPHL. Major criteria include (1) more than 4 yellow dots in 4 images in the frontal area; (2) lower average hair thickness in the frontal area compared with the occiput; and (3) more than 10% of thin hairs (<0.03 mm) in the frontal area. Minor criteria include (1) increased frontal to occipital ratio of single-hair pilosebaceous units; (2) vellus hairs; and (3) perifollicular discoloration. The diagnosis of FPHL is made during the presence of two major criteria or one major plus two minor criteria.

7.6. Pull Test. Pull test is a noninvasive diagnostic technique, very easy to perform and to repeat. Pull test is very helpful to rapidly determine the ongoing activity and severity of any kind of hair loss. Briefly, a bundle of about 50–60 hairs is grasped between the thumb, index finger, and middle finger from the base near the scalp. The hair is firmly, but not forcibly, tugged away from the scalp as fingers slide along the hair shaft [47]. The test is positive when more than 10% of the grasped hair (in average more than six hairs) can be pulled out [48]. If fewer than six hairs can be easily pulled out, this is considered normal physiologic shedding [49]. The test has a large interobserver variation and can be influenced by cosmetic habits, hair manipulation, and shampooing. Each clinician has to standardize his own procedure. The pull test should be done in all the scalp areas: right and left parietal, frontal, and occipital regions. A positive test present in more than one scalp region can be seen during a telogen effluvium. The patients suffering from FPHL may have a positive pull test only during the active phases in the affected area. A diffuse positive pull test requires always further investigation to exclude telogen effluvium [23].

8. Other Diagnostic Techniques

8.1. Scalp Biopsy. Scalp biopsy is an essential instrument in the diagnosis of cicatricial and selected forms of noncicatricial alopecia [50]. Although scalp biopsies are usually not needed to diagnose FPHL, they can be helpful if the clinical evaluation does not provide a definitive diagnosis, for example, when scalp changes suggestive of cicatricial alopecia of diffuse alopecia areata are present. A 4 mm punch extending into the subcutaneous fat should be performed on the central scalp area. It is best to avoid the bitemporal area as this region may have miniaturized hairs in women without hair loss [23]. Scalps biopsies should be read by experienced dermatopathologists using both vertical sectioning and horizontal sectioning. Horizontal sections allow a rapid evaluation of hair follicle number, diameter, grouping, and morphology maximizing the diagnostic yield [51]. In FPHL, there are an increased number of miniaturized (vellus-like) hairs. The ratio of terminal to vellus-like hair follicles is typically >3 : 1 in women suffering from this condition against >7 : 1 in the normal scalp [14]. Other typical histopathological features are an increase of telogen : anagen ratio and an increased number of follicular stelae. A mild perifollicular inflammation around the upper portion of hair follicle as well as perifollicular fibrosis may also be seen [52].

8.2. Trichogram. The trichogram is a semi-invasive (plucking) microscopic method for hair root and hair cycle evaluation. The term "trichogram" was given by Pecoraro et al. in 1964, who described further trichometric parameters such as hair shaft diameter, hair growth, and telogen rate [53]. The trichogram is based on the hair cycle and quantifies hair follicles in their different growth phases: anagen, telogen, or catagen. Trichogram may be recommended in individual cases of FPHL if another diagnosis is suspected like an anagen-dysplastic effluvium or a loose anagen syndrome. Trichogram can also be useful as a complementary element that may confirm an early diagnosis of FPHL by showing inhomogeneous hair shafts. However, a recent study suggests that dermatoscopy is more useful for the diagnosis of FPHL than trichograms [54].

8.3. Phototrichogram/TrichoScan. Phototrichograms are automatic digitalized imaging techniques used to examine features of hair loss for diagnosis and followup [55]. A small area of the scalp is trimmed and followed with imaging. The proportion of anagen, telogen, and shed hairs as well as the rate of growth and the density can be recorded and compared [56]. The FPHL is characterized, like AGA, by a decrease in the frontal hair density compared with the occipital density. To ensure reproducibility tattoos identifying the studied area are required [48]. When available, those techniques are helpful for long-term followup and quantification, but currently they are mainly used as a tool for clinical studies [57].

8.4. Laboratory Tests. Ferritin and thyroid-stimulating hormone levels may be measured, especially in diffuse effluvium. The association between FPHL and low ferritin levels was suggested in two different studies [58, 59] which reported significantly lower ferritin levels in women suffering from FPHL compared with controls. More recent studies have not shown sufficient evidence of the relationship between low ferritin level and FPHL and do not recommend iron supplementation in the absence of deficiency anemia [60]. In 2008, Bregy and Trüeb even suggest no association between iron deficiency and hair loss in women [61]. The experts agreed that an extensive endocrinological investigation is not

necessary in all women. Research for hyperandrogenic state will be performed in women with FPHL when the history and the clinical examination are suggesting an androgen excess (e.g., hirsutism, irregular menses, acne, and galactorrhea). Some authors speculate that the Hamilton IV pattern is more common in women suffering from ovarian hyperandrogenism, but there are no studies to corroborate or invalid this theory. The authors recommend a free androgen index test (FAI = total testosterone ($nmolL^{-1}$) × 100/sex hormone-binding globulin (SHBG)) and prolactin as screening parameters for ovarian hyperandrogenism and dehydroepiandrosterone sulfate (DHEAs) and 17-hydroxyprogesterone (17-OH) as screening parameters for surrenal hyperandrogenism [23, 39, 62]. Depending on the results, further investigations may be needed and an interdisciplinary approach involving gynecologists, endocrinologists, and dermatologists may be required. Note that androgen levels testing should be done during the follicular phase, between the fourth and the seventh day of the cycle and that oral contraceptives should be discontinued for at least two months prior to this testing [63]. The patients should be informed that cessation of contraception may induce a three-month lasting telogen effluvium.

9. Summary

FPHL is a very common, nonscarring form of hair loss that can occur in all ages but most commonly in postmenopausal women. Although hormonal factors and genetic predisposition are believed to contribute to FPHL, the complete mechanism remains elusive and the most affected women have normal androgen levels. FPHL does not cause physical discomfort but the hair loss can contribute to significant psychological distress. Generally, the condition is clinical diagnosis, suggested by the reduction in hair density with a characteristic distribution. Depending on patient history and clinical evaluation, further diagnostic tests may be required.

Disclosure

Thierry Huart is Medical Illustrator.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Pruritus in Female Patients

Julien Lambert

Department of Dermatology and Venereology, University Hospital Antwerp/University of Antwerp, Wilrijkstraat 10, 2650 Edegem, Belgium

Correspondence should be addressed to Julien Lambert; julien.lambert@uza.be

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Pruritus is a frequent symptom in many dermatological diseases. In this review we want to focus on not only itch problems specific to women, namely, pruritic vulvodermatoses, but also the specific pruritic dermatoses of pregnancy. The specific characteristics of the vulva and the hormonal changes during the different age periods make these dermatoses very particular. It seems that vulvar diseases are still underdiagnosed and undertreated. Pruritic vulvar diseases have a huge impact on quality of life. The most common pruritic diseases will be discussed, such as atopic and contact dermatitis, psoriasis, lichen sclerosis, lichen planus, and infectious vulvaginitis. We focus on the diagnostic issue of these diseases and will consider the general principles of therapy.

1. Introduction

Pruritus is a frequent symptom in many dermatological diseases. In this review we want to focus on not only itch problems specific to women, namely, pruritic inflammatory vulvar dermatoses, but also the specific dermatoses of pregnancy. Considering these dermatoses we have to take into account the following points: the distinct epithelial characteristics of the vulva in its different regions, the temporal hormonal shifts that lead to cyclic changes in the skin's basic composition, and finally the presence of estrogens receptors on keratinocytes. The changing level of estrogens leads to changes in hydration, collagen content, and concentration of glycosaminoglycans. In addition there will be also changes in vulvovaginal pH and microflora compositions [1].

Vulvovaginal pH is high in childhood, but in puberty the pH starts to decrease from an average of 7 to an average of 4 in adult women. *Lactobacilli* start to colonize the vulvovaginal area. In the first half of the hormonal cycle estrogen levels rise and vulvovaginal epithelial cells proliferate. In the second half of the cycle, which is progesterone mediated, the keratinocytes desquamate. There are also changes of the bacterial flora during the hormonal cycle. Also the pH levels are going to fluctuate and eventually cause pruritus; an increase of pH may activate the proteinase-activated receptor-2 (PAR-2) which is a well-known itch mediator. Due to the decrease of estrogens, vaginal pH is going to rise in menopause [1].

2. Atopic and Contact Dermatitis

The commonest vulvar dermatosis in both adults and children is dermatitis. The majority of these patients are atopic [2]. In prepubertal girls atopic and irritant dermatitis occurs often together. Clinical examination shows erythematous and scaly labia majora with frequently rugosity, due to lichenification. The labia minora may be erythematous and scaly. The itch is constant, and the dermatosis is fluctuating. Irritant contact dermatitis may be due to poor hygiene habits or excess use of soap or prolonged wearing of wet swimming suits. Allergic contact dermatitis is very unusual in children because the exposition to potential allergens is low [2].

In adult women allergic and irritant contact dermatitis accounts for around 50% of cases of chronic vulvovaginal pruritus [3]. It may complicate the presentation of other dermatoses. Theoretically there is an increased risk for sensitization: because of differences in structure, occlusion, and hydration and susceptibility to friction, vulval skin is more permeable than exposed skin [4]. The predominant symptom is itch, but burning and pain may also be present, especially if fissures occur. Clinical examination shows erythema and

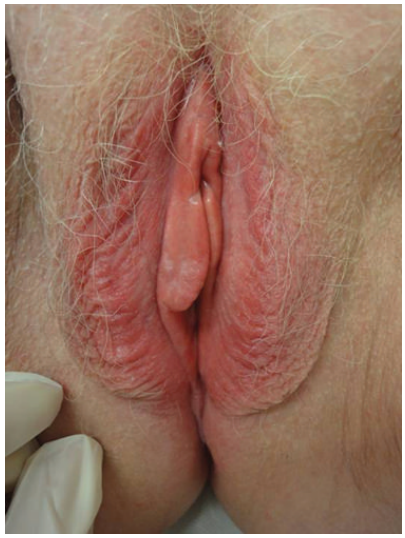

Figure 1: Allergic contact dermatitis to an intimate product.

swelling and in chronic cases lichenification is frequently present (Figure 1). Common irritants include soaps, antiseptics, lubricants, spermicides, tampons, sanitary pads, and synthetic underwear. Several studies have highlighted the usefulness of patch testing in case of vulval pruritus [4–6]. A prospective study showed a very high rate of contact sensitivity in patients presenting with vulval pruritus. One or more clinically relevant allergens were found in 44% of the subjects tested [5]. Many relevant allergens did not belong to the European standard series so there is a need for extended patch testing. Interesting to note is that a study demonstrated that 47% of patients with lichen sclerosus had positive patch tests [7]. Topical anesthetics and antibiotics, preservatives, dyes, and perfumes are potential allergens. In order to get a complete list of all the topical applications that women use, do take into account that women regularly use preparations available over the counter. Excessive cleansing of the vulvar skin, as well as urinary and fecal incontinence, may also precipitate to an irritant dermatitis. Finally estrogen-deficient patients are particularly prone to irritant contact dermatitis [8]. Management consists in the removal of all irritants and potential allergens and application of topical steroids until the skin returns to normal.

3. Lichen Sclerosus

Lichen sclerosus (LS) is a chronic inflammatory dermatosis of unknown etiology first described by Hallopeau in 1897 as an atrophic form of lichen planus [9]. Most cases are seen in prepubertal girls or in postmenopausal women. A possible association with psoriasis has been suggested [10]. The classical presentation is a "figure- of-eight" shaped white plaque around the vulva and anus. Classically it is taught that LS does not affect the vagina, in contrast to lichen planus, which is an important clue in the differential diagnosis. A few cases of LS with vaginal involvement have been reported [9]. Atrophy, erosions, fissures, and ecchymoses may also be present. In advanced cases a loss of genital architecture may occur with subsequent effacement of the labia minora and clitoris (Figure 2). In most cases the itch is predominant but some women will complain more of soreness, burning, and pain. Some pediatric cases resolve with puberty, while others may continue to adulthood. The authors who presented very recently the 2 new cases of LS with vaginal involvement put the question forward if this is not underdiagnosed because the vagina may not be examined carefully for LS or because lesions may be subtle or atypical. Both presented cases had significant pelvic organ prolapse and so the vaginal mucosa was more chronically exposed. This brings into question whether squamatization of the vaginal mucosa may play a role in the development of the vaginal LS lesions [9]. The risk of developing squamous cell carcinoma in longstanding cases of lichen sclerosus is 5% or less. Treatment consists of high potency topical corticosteroids, also in younger patients; however, it is proposed to use not the most potent preparations in these younger patients.

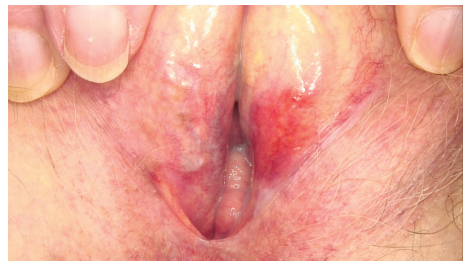

Figure 2: Lichen sclerosus in a postmenopausal woman.

4. Lichen Planus

The prevalence of lichen planus (LP) in the genital area is much lower than lichen sclerosus [11]. Differential diagnosis with lichen sclerosus is not always easy. Lichen sclerosus is normally confined to the vulva while lichen planus may affect the vulva as well as the vagina. Other localizations such as the scalp, oral mucosa, skin, and nails may help to confirm the diagnosis of lichen planus. Women complain of soreness, itching, burning, and dyspareunia. Three types of vulvar lichen planus have been described: erosive, classical, and hypertrophic [12]. Erosive lichen planus is the far most common variant (85% of the cases). Erosive LP is characterized by erosions involving the introitus, clitoris and clitoral hood, labia minora and majora. A lacy white edge to the erosions is regularly seen. Healing erosions may appear as a glazed erythema. Vaginal involvement is very common and presents with vaginal erythema, contact bleeding, erosions, and scarring with synechiae. In rare cases vaginal lesions may be the only manifestation. Very recently diagnostic criteria for erosive LP of the vulva have been published [13]. The classical type presents with small purple, polygonal papules, with sometimes a reticulate lace pattern. Postinflammatory hyperpigmentation is rather frequent in the flexures. Hyperkeratotic lichen planus presents as single or multiple white-hyperkeratotic papules and plaques. Many

patients present with a mix of different clinical subtypes. A very recent study documents that a significant percentage of patients with vulval LP have associated lichen planopilaris [14]. The commonest pattern of scalp lichen planopilaris was that of the frontal fibrosing alopecia variant (FFA). All of these FFA patients also had oral LP. Treatment consists in the first place of topical steroids. Classical LP is normally treated with a moderately potent topical corticosteroid. For hypertrophic disease a very potent topical corticosteroid is indicated. A hypertrophic lesion that responds poorly to treatment requires a biopsy to rule out a malignant lesion. Erosive vulvovaginal lichen planus is difficult-to-treat dermatosis, which is usually chronic and persistent. Systemic therapy has to be taken into consideration if local therapy is insufficient. Because of the rare risk of squamous cell carcinoma, a long term follow-up is necessary.

5. Lichen Simplex Chronicus

The clinical presentation of lichen simplex chronicus is also typical on the vulva: the skin is thickened, lichenified, and often hyperpigmented due to chronic rubbing and scratching of the skin. Lichen simplex chronicus may occur secondary to pruritic conditions such as lichen sclerosus or contact dermatitis. It is also important to take neuropathic itch into consideration as etiology of lichen simplex chronicus. This could be associated with sacral spinal compression, postherpetic neuralgia, and diabetic neuropathy [1].

6. Psoriasis

Psoriatic lesions on the vulva are more common in children than in adults. There is no difference in the clinical presentation of psoriasis of the vulva in children and adults. In babies it may initially present as napkin psoriasis. Clinical examination shows itchy well-demarcated symmetric red plaques without scaling in the vulvar and perianal regions. The vagina is spared. Genital itch in psoriatic women is very common. A Polish study revealed a high prevalence of vulvar itching and or burning in women with psoriasis. Moreover this vulvar discomfort and accompanying psoriasis had a significant influence on the psychosocial wellbeing of the patients [15]. Treatment is similar in adults and children and consists of moderated-to-potent topical corticosteroids.

7. Infectious Vulvovaginitis

In prepubertal girls a group A betahemolytic streptococcal infection can cause vulvar symptoms. In the acute form there is a sudden onset of an erythematous swollen painful vulva and vagina with a thin mucoid discharge. The subacute form presents as pruritic erythematous patches and plaques in the vulvar and perianal regions [2]. These infections are diagnosed by vaginal and perianal swabs. The origin of the infection is thought to be a pharyngeal infection; however, clinical signs are not always present. Treatment consists of oral penicillin or amoxycilline. Pinworm is a common cause of vulvar and perianal pruritus in children. It may be associated with eczematous lesions and is treated with mebendazole.

Vulvovaginal candidiasis does not occur normally before menarche. On the contrary, many women of reproductive age experience one or more episodes of vulvovaginal candidiasis. Vulvovaginal candida colonization occurs in at least 20% of all women. It is an estrogen dependent process, so it occurs almost exclusively in the reproductive years, especially in the premenstrual period, when hormone levels are high.

Pregnancy, antibiotic use, hormonal contraceptive medication and hormone replacement therapy, and tamoxifen may increase estrogen levels and could be responsible for more frequent colonization and infections [1]. Also changes in the immune system, such as diabetes, HIV, thyroid disease, lupus, and corticosteroid use can cause yeast infections. Not all patients at risk develop Candida infections. Genetic variation plays an important role in host susceptibility. Common polymorphisms in genes of the immune system have been associated with recurrent vulvovaginal candidiasis [16]. Patients complain of itching and burning of the vulva and also a white discharge and vulvovaginal redness. Reliable diagnosis is based on the correlation of clinical features with mycological evidence [17]. In most of the cases, Candida albicans is responsible. An asymptomatic colonization does not need to be treated, except in case of immunosuppression or chronic recurrent vulvovaginal candidiasis [18]. For the treatment of an acute vulvovaginal candidiasis, polyenes, imidazoles, or ciclopiroxolamine in local therapy are proposed or oral triazoles for 1 to 6 days [18]. In case of chronic recurrent *C. albicans* vulvovaginitis the best results are obtained with an individualized decreasing-dose maintenance fluconazole regimen [19]. A German recommendation proposes a prophylactic local treatment of asymptomatic vaginal candida colonization during the last 6 weeks of pregnancy to protect the baby during vaginal delivery. A significant reduction of neonatal candida infection rates was observed [18]. Other species such as *C. glabrata*, *C. tropicalis*, and *C. parapsilosis* may sometimes occur and they are much more difficult to treat.

8. Pruritus in Pregnancy

Pruritus is the main dermatological symptom in pregnancy, which is a very particular hormonal period in a woman's life. Pruritus is also a very prominent symptom of the specific dermatoses of pregnancy.

The most recent classification includes pemphigoid gestationis, polymorphic eruption of pregnancy, intrahepatic cholestasis of pregnancy, and the new entity atopic eruption of pregnancy, a new "umbrella" concept comprising atopic dermatitis in pregnancy, prurigo of pregnancy, and pruritic folliculitis of pregnancy [20].

8.1. Polymorphic Eruption of Pregnancy. Polymorphic eruption of pregnancy previously known as pruritic urticarial papules and plaques of pregnancy occurs in the latest pregnancy weeks or immediately postpartum. It is associated with primigravida, excessive maternal weight gain, and multiple

pregnancies [21]. The pathophysiology is unknown, but a relationship with damage of the collagen fibers due to distension and overstretching of skin is suspected [22]. The clinical examination shows in the beginning urticarial papules and plaques and later on a polymorphous aspect is seen in more than 50% of the patients with vesicular, targetoid, and eczematous lesions. These lesions start within the striae distensae on the abdomen and spread to the buttocks and the proximal extremities. The rash spares very typically the umbilical region. The rash generally resolves within 6 weeks. Recurrences are very rare and are only reported in case of multiple pregnancies. Histopathology is not specific, so normally diagnosis is made by clinical picture and history. Treatment consists of topical corticosteroids with or without antihistamines.

8.2. Pemphigoid Gestationis. Pemphigoid gestationis, formally known as herpes gestationis, is a rare bullous autoimmune disease, which normally occurs in the second half of the pregnancy or immediately postpartum. The pathogenesis of this disease is based on the production of circulating immunoglobulin G antibodies that bind to bullous pemphigoid antigen 2 (BP-180) in the hemidesmosomes of the dermoepidermal junction, which results in the damage of the membrane and the production of tense bullae. Clinical examination shows typically tense bullae like in bullous pemphigoid in the neighbourhood of urticarial lesions. The lesions start on the abdomen and do not spare the umbilical region and there is no association with the striae distensae. The lesions may involve the total body, but there is no mucosal involvement. The diagnosis is confirmed by histology and especially direct immunofluorescence which shows a linear C3 along the dermoepidermal junction. Pemphigoid gestationis tends to resolve within weeks to months of delivery. There is a higher risk of premature and small-for-gestational age babies [22]. Treatment consists of antihistamines and systemic corticosteroids.

8.3. Intrahepatic Cholestasis of Pregnancy. Intrahepatic cholestasis of pregnancy is a condition that has not always been included in the classifications of pregnancy dermatoses because it is not associated with primary skin lesions.

Patients present secondary skin lesions caused by scratching. It is a hormonally triggered reversible cholestatis, occurring in late pregnancy in genetically predisposed women. The incidence in Europe is much lower than in South-America [23]. The pathogenesis is characterized by an inability to excrete bile salts, causing elevated serum bile acid levels, responsible for pruritus in the mother and influencing negatively the fetal prognosis. There is an increased risk of prematurity, intrapartal fetal distress, and stillbirth.

Patients present a sudden-onset pruritus that starts in the palmoplantar regions but becomes very quickly generalized to the entire body. Due to scratching and rubbing, patients present secondary linear excoriations and prurigo nodularis lesions on the extensor surfaces of the arms and legs. Signs of icterus are seen in approximately 10% of the cases. Diagnosis is made by the rise of serum bile acid levels > 11 μmol/L [22]. Normal levels are 6 μmol/L, but during pregnancy 11 μmol/L is tolerated. Liver function tests are normal in 30% of the cases. The elevation of the serum bile acid levels has a prognostic value; in case of levels of >40 μmol/L, the fetal risk is markedly higher [24]. Treatment consists of ursodeoxycholic acid which reduces serum bile acid levels. This treatment reduces maternal pruritus and also fetal prognosis. Recurrences occur in next pregnancies and in case of oral contraceptive treatment.

8.4. Atopic Eruption of Pregnancy. Ambros-Rudolph et al. introduced the new term atopic eruption of pregnancy in 2005 to cover all patients formerly given diagnosis of eczema of pregnancy, prurigo of pregnancy, and pruritic folliculitis of pregnancy [20]. A prospective study on pruritic skin diseases in pregnancy had demonstrated a higher prevalence of atopic eczema. This finding was not taken into consideration in former classifications. They observed a considerable overlap among patients with eczema of pregnancy, prurigo of pregnancy, and pruritic folliculitis, both clinically and histopathologically, so they grouped them within a new disease complex "atopic eruption of pregnancy." There still exist controversies regarding this terminology [25].

This is the most common pruritic condition in pregnancy noted in almost 50% of the patients. Only 20% of the patients suffered from exacerbation of a pre-existing atopic dermatitis as 80% experienced atopic skin lesions for the first time during their pregnancy [20]. These eczematous lesions could be related to the typical dominance of the Th-2 immunity observed during pregnancy. In order to prevent fetal rejection, normal pregnancy is characterized by a lower Th-1 cytokine production and an enhanced Th-2 cytokine production [26]. Atopic dermatitis is considered to be a Th-2 dominant disease. The Th-2 shift associated with pregnancy may explain the exacerbation of atopic dermatitis during pregnancy. In contrast to the other specific dermatoses of pregnancy the onset occurs in 75% of the cases before the third trimester. The skin lesions can be divided in either eczematous type skin (E-type) changes or prurigo type lesions (P-type) [20]. The eczematous type lesions are located in the classical localizations like the face, the neck, the presternal region, and the flexure sides. The prurigo lesions occur on the extensor surfaces of the extremities. Elevated serum IgE levels are present in 30 to 70% of the cases [22]. Fetal prognosis is unaffected. Recurrences in later pregnancies are to expect. The treatment consists in the first place of topical corticosteroids. In severe cases, systemic corticosteroids, antihistamines, and ultraviolet B phototherapy may be considered.

8.5. Treatment during Pregnancy. Treating pruritic diseases in pregnancy remains frequently a challenge. Most of the time topical corticosteroids and antihistamines will be treatment of choice. Little is known about the effects of local corticosteroids on the fetus. A recent European evidence-based guideline suggests the following recommendations [27]. Mild/moderate topical corticosteroids are preferred to more potent corticosteroids. Potent/very potent local

Table 1: Frequent pruritic dermatoses during different age groups.

Prepubertal	Reproductive age	Postmenopausal
Atopic dermatitis	Atopic dermatitis (LF)	
	Allergic contact dermatitis	Allergic contact dermatitis (LF)
Irritant contact dermatitis	Irritant contact dermatitis	Irritant contact dermatitis
Psoriasis	Psoriasis	Psoriasis
Lichen sclerosus	Lichen sclerosus (LF)	Lichen sclerosus
Streptococcal infection	Vulvovaginal candidiasis	
	Lichen simplex	Lichen simplex
		Atrophic vulvovaginitis

LF: less frequent than in the other age groups.

corticosteroids should be used as second-line therapy as short as possible and meanwhile appropriate obstetric care should be provided because there is an increased risk of fetal growth restriction. A very recent study showed a significantly increased risk of low birth weight in case that more than 300 g of potent or very potent topical corticosteroids during the entire pregnancy was applied [28]. There are no data available to determine if newer lipophilic topical corticosteroids (mometasone furoate, fluticasone propionate, and methylprednisolone aceponate) are associated with a lower risk of fetal growth restriction. On theoretical grounds they have a more favourable side-effect profile. Systemic corticosteroids have a greater potential for fetotoxicity than local corticosteroids because of a greater bioavailability. They are associated with a reduction in fetal birth weight and an increase in preterm delivery.

There is also a lack of knowledge concerning the use of antihistamines during pregnancy. The older, sedating antihistamines such as dimethindene and clemastine are considered as safe because they are already prescribed for very long time [29]. Regarding the use of hydroxyzine during the first trimester, reports concerning a slight higher risk of malformation [30] and risk of neonatal seizures in case of use in late pregnancy [31] invite cautiousness. The antihistamines of the second generation, such as cetirizine, loratadine, fexofenadine, desloratadine, and levocetirizine provoking low or no sedation, are categorized as medications of which we do not have extensive information about use in humans but animal studies could not show evidence of embryotoxicity or teratogenicity [29]. Loratadine and cetirizine are among the second generation antihistamines the ones best studied. They can be prescribed after the first trimester in case of well-considered indications. Administration just before or after the birth has to be avoided.

9. Atrophic Vulvitis

Atrophic vulvitis is a common complaint of postmenopausal women. Estrogens have a proliferative influence on the vulvovaginal epithelium and enhance the circulation and the hydration of the skin and connective tissue [32]. The decrease in estrogens is responsible for a thinner epithelium, a loss of turgor, and a decline of the fat depots of the labia majora. The skin becomes vulnerable and dry and is atrophic, erythematous, and desquamative. Patients complain of itching and a burning sensation. Due to loss of glycogen in the vulvar epithelium the colonization of *lactobacilli* is decreased. *Lactobacilli* produces lactic acid from glycogen and produces so an acid pH [32]. A higher pH creates a favorable environment for pathogenic organisms. The treatment consists in the first place of topical estrogen therapy. The systemic resorption is negligible.

10. Conclusion

It is only the last years that there are an increasing number of publications on female specific pruritus. Girls and women may experience during the different age groups a series of pruritic dermatoses as shown in Table 1. These diseases may have a high impact on quality of life. It is therefore of outmost important to recognize them early and to treat them adequately. We have still the impression that until now these specific female itch entities are underdiagnosed. Finally, we focused on inflammatory diseases. Nevertheless, we want to mention briefly that especially in elderly women always malignant lesions have to be taken into consideration in the differential diagnosis of pruritic vulvar diseases.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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Review Article

Streamlining Cutaneous Melanomas in Young Women of the Belgian Mosan Region

Trinh Hermanns-Lê[1,2] and Sébastien Piérard[3]

[1] *Department of Dermatopathology, Unilab Lg, University Hospital of Liège, 4000 Liège, Belgium*
[2] *Dermatology Unit, Diagnostic Centre, 4800 Verviers, Belgium*
[3] *INTELSIG Laboratory, Montefiore Institute, University of Liège, 4000 Liège, Belgium*

Correspondence should be addressed to Trinh Hermanns-Lê; trinh.hermanns@chu.ulg.ac.be

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Sporadic cutaneous melanoma (SCM) has shown a dramatic increase in incidence in Caucasian populations over the past few decades. A particular epidemiological increase was reported in women during their childbearing age. In the Belgian Mosan region, a progressive unremitting increase in SCM incidence was noticed in young women for the past 35 years. The vast majority of these SCMs were of the superficial type without any obvious relationship with a large number of melanocytic nevi or with signs of frequent and intense sunlight exposures as disclosed by the extent in the mosaic subclinical melanoderma. A series of investigations pointed to a possible relationship linking the development of some SCM to the women hormonal status including the effect of hormonal disruptors. These aspects remain, however, unsettled and controversial. It is possible to differentiate and clearly quantify the SCM shape, size, scalloped border, and variegated pigmentation using computerized morphometry as well as fractal and multifractal methods.

1. Introduction

The sporadic cutaneous melanoma (SCM) incidence has risen over the past decades across various groups of Caucasian populations [1–3]. Such an increase is possibly genuine or caused, in part, by intensive population screenings in individuals of light complexion and by early detection procedures. A large number of nevi are apparently the strongest risk factor for SCM in Caucasians [4]. The numerical density in nevi is a heritable characteristic with about 60% of the variation attributed to other additional genetic effects. Fair presentations of skin and hair represent a combination of risk factors whose magnitude remains much smaller. Different risks to develop SCM are associated with the phenotypes. They include presence of photodamage such as solar elastosis and actinic keratoses [5]. Globally, repeat sun exposures are linked to an increased SCM incidence with decreasing latitude. It remains that this type of environmental exposure is difficult to quantify. In addition, such an association is in part confounded by the fact that population screening in geoclimatic regions with high intensity of sunlight exposure commonly increases the detected SCM incidence.

This review is focused on gender differences in SCM incidence according to age. Both computerized morphometry and fractal analysis will be applied to the clinical aspect of the neoplasms and to the peritumoral apparently uninvolved skin.

2. Gender Influence and Age-Related Melanoma Incidence

In general, cancers sensitive to female sex steroids are associated with several risk factors, such as low parity, infertility, early age at menarche, and late age at menopause [4]. As far as SCM is concerned, the neoplasm develops predominantly in white people and is under gender influence [1, 6–10]. The SCM incidence rates are particularly high in Northern Europe, North America, and Australia. By contrast, they are low among the indigenous populations of Africa, Asia,

Latin America, and Southern Europe. About one-third of all SCM in women develop during their childbearing age particularly between 25 and 29 years [11, 12]. Until the age of 45 years, SCM incidence rates in women exceed those in men [2, 3]. Such a trend is levelled off later [1]. This trend suggested the possible intervention of some hormonal influences [13–19]. Investigations were previously focused on women endocrine status including the possible impact of oral contraceptives, hormone replacement therapy, age at first child, age at menarche, age at menopause, menopausal status, and administration of fertility drugs [20–23]. They reflected potential influences of exogenous and endogenous hormones. In particular, a significant increase in SCM risk was reported for late age at first birth. By contrast, women with several children appeared to be at lower risk for SCM. These epidemiological findings were apparently linked to a series of socioeconomic confounders. Some concerns originated from pregnancy-related SCM, reports on skin hyperpigmentation during oral contraception, and nevi enlargement and darkening during pregnancy [17]. However, the link between SCM occurrence and pregnancy and hormonal and reproductive factors remains controversial. By contrast, gender is an established prognostic factor in SCM with women having a better overall prognosis than men [10].

The influence of fertility drugs on SCM risk has not been extensively studied despite the common use of this group of exogenous hormones over the last decades [21] and their established effect on ovulation and endogenous hormone production. Given the increasing frequency of prescriptions of fertility drugs among infertile couples, the concern about fertility drugs increasing the SCM risk has been considered as important public health worries. The impact of hormonal disruptors has been recently evoked [24–29]. They could interfere with estrogen receptors and alter the functions of some HOX genes [29, 30].

The pattern of age-incidence rates of SCM in women resembles that of breast cancer. They are higher in women than in men especially before 45 years of age. Afterwards, differently from men, rates of increase slow down [31]. Furthermore, a higher risk of breast cancer among women with a history of SCM and excess SCM risk among breast cancer patients have been reported in several studies [32–34].

3. Clinical Presentation

SCM exhibits several clinical presentations [35]. In our experience, these neoplasms developed in young women are generally flat and slow growing [29]. Of note, clinically featureless SCM is possibly disclosed using computerized monitoring. Basically, the clinical screening for SCM relies on the ABCDE rules in which A denotes asymmetry, B indicates border irregularity, C denotes color variegated pattern, D stands for diameter, and E indicates the evolutionary extension in size [36]. Other features are added to the ABCDE acronym, including changes in shape, shades of color, symptoms (itch, tenderness), and surface presentation (e.g., bleeding, crusting, scaling, etc.). They allow detection of smaller and morphologically featureless SCM.

The diagnosis of SCM in its early phase of development is mandatory in order to improve the prognosis and decrease mortality. For this purpose, increased interest has been paid to cyanoacrylate skin surface strippings (CSSS) [37, 38] and dermoscopy [39, 40].

CSSS is a minimally invasive method exclusively collecting the superficial layers of the stratum corneum. In melanocytic neoplasms, melanin is present inside corneocytes and eventually in atypical melanocytes. Melanin restricted only inside corneocytes is a feature of benign neoplasms such as lentigines and melanocytic nevi. By contrast, the presence of atypical melanocytes inside the stratum corneum is strongly suggestive of SCM but also, in rare instances, of a benign melanoacanthoma [37]. Thus, CSSS proves to be sensitive and specific for distinguishing SCM from benign melanocytic tumors such as common melanocytic nevi, dysplastic nevi, or pigmented seborrheic keratoses. For research purposes, karyometry of neoplastic melanocytes is conveniently performed on CSSS [38].

Dermoscopy (surface microscopy, epiluminescence microscopy) is a simple optical method leaning on incident light magnification and pattern analysis. It allows identification of morphological aspects invisible with the naked eye. Currently, there is a suggested two-stage procedure for the diagnosis of pigmented skin lesions using dermoscopy [39, 40]. The first stage permits the differentiation of melanocytic tumors from nonmelanocytic neoplasms including seborrheic keratoses, pigmented basal cell carcinoma (BCC), and hemangioma. Once a melanocytic tumor is diagnosed, the second stage allows differentiation of SCM from benign melanocytic tumors such as lentigo simplex, typical and atypical nevi, and solar lentigo.

Conventional dermoscopy does not detect all in situ SCM [41, 42]. Several descriptors characterizing in situ and thin SCM have been proposed [43, 44]. It remains that classification into different SCM subtypes is not immediately recognizable according to the established main dermoscopic patterns [45].

4. Computerized Monitoring

From two-dimensional images of pigmented tumors, a series of feature operators are conveniently applied to extract texture descriptors useful for clinical diagnosis [46]. Computerized monitoring devices record clinical presentations and/or digital dermoscopy images [47]. They allow tiling on the computer screen for comparison of pigmented lesions for change over time. A number of devices using a variety of cameras have ideally to be controlled by adequate calibration. A series of semiautomatic algorithms have been designed. The attractiveness of computerized monitoring is the rationale of clinical decision making. When a melanocytic tumor remains unchanged, it is assumed to be benign, and when it has changed, excision is warranted for concern about malignancy. Undoubtedly, a preliminary diagnostic procedure avoiding unnecessary excisions is preferable. The procedures are conveniently divided into two categories corresponding to short-term and long-term monitoring, respectively, [48–50].

Short-term monitoring, usually over a 3-month period, is used to establish a clinical judgment about abnormal melanocytic tumors that do not exhibit clear SCM features [49, 51]. These lesions usually belong to two groups, namely, moderately atypical melanocytic nevi and skin melanocytomas that apparently remained unchanged over time and discretely atypical melanocytic nevi exhibiting features of change. In the short-term monitoring procedure, any variation in presentation over a 3-month period, except increased or decreased milia-like cysts or overall variation in pigmentation consistent with increased or loss of tan in the surrounding skin, requires excision of the neoplasm. The specificity of the computerized morphometry for SCM reaches about 80%, and the sensitivity is near 100%, although such estimate has not been conclusively demonstrated [46–48].

Computerized short- and long-term monitorings help identifying featureless-appearing SCM that can only be detected by high resolution morphological changes. Some of these featureless SCM are conveniently demonstrated by short-term monitoring [51, 52]. Monitoring investigations raised concerns about the diagnostic accuracy of early SCM in regular clinical setting [46].

Both short- and long-term computerized monitoring readily allows detection of some featureless SCM, but the physician efficiency is subjected to interindividual differences. Long-term monitoring allows comparison of atypical melanocytic tumors over prolonged surveillance periods. These monitored atypical tumors are not considered as possible SCM at the time of imaging. Such diagnostic procedure is generally restricted to patients who have multiple dysplastic nevi [48] or enlarging compound nevi under human growth hormone therapy [53]. About 5% of pigmented neoplasms monitored show obvious changes over a 12-month surveillance period [50]. The changes correspond to tumor enlargement, alterations in shape or color, regression, and appearance of dermoscopic features SCM.

Melanocytic nevus stability depends on the age of the patient under monitoring. Although about 15% of regular nevi show prominent changes before 20 years, only about 2% of melanocytic nevi in adults older than 40 years show similar changes [54]. Such age relationship was further reported when monitoring atypical nevi, with about 10% of them changing in patients younger than 28 years of age but in only 3% of adults older than 48 years [55]. Hence, long-term computerized monitoring is particularly efficient in adults older than 40 years [46].

5. Computerized Morphometry and Fractal Analysis of the Clinical Aspect

Many biologic processes are known to be heterogeneous, especially in the oncologic field. Repartition of estrogenic receptors is inhomogeneous, and the heterogeneity of these variables is deduced from multiple measures sampled on different sites of the tumor. Although such evaluation does not constitute a quantitative approach, it evokes the heterogeneity of the phenomenon.

The actual size, shape, and symmetry of pigmented lesions are possibly assessed using computerized morphometry [48, 49]. The optical image of excised SCM specimens is acquired using a video camera and image analysis following a morphometric computer program. Both the SCM area and form factor (Form AR) are measured. Form AR identifying the fine irregularities of contour ranges from 0 to 1. Values close to 1 indicate a smooth and regular outline. The area can be changed to a circle the diameter of which (D circle) is calculated.

When data do not exhibit a Gaussian distribution, the Kolmogorov-Smirnov test and the Mann-Whitney U test are conveniently used for evaluating the differences in distributions and medians of the values. Multivariate discriminant analysis demonstrates the ability of the analytic variables to discriminate the vast majority of the melanocytic neoplasms, particularly when two-dimensional variables are included [47].

One of the major clinical diagnostic criteria for SCM is expressed by morphometry as the combination of a large D circle value and a small Form AR. These two independent parameters are combined in a ratio to define the clinical index of atypia (Ia) corresponding to $\text{Ia} = \text{D circle} (\text{Form AR})^{-1}$. It is higher when the D circle is large and Form AR is small, lower than the value 1 [49].

The variegated SCM aspect is possibly analysed using fractal analysis because the fractal concept of self-similar structures is applicable to clinical pictures of some skin neoplasms [56–58]. Optical images are commonly acquired using a video camera and digitized on a matrix of 512×512 pixels with 256 gray levels. A texture analysis on the gray levels of the SCM images is conveniently performed by means of fractal characterization using both fast Fournier spectrum and multifractal analysis [57]. Contiguous optical fields are observed and measurement data are averaged before using specific algorithms [57]. Enhancement of local discontinuities or edges is performed using a gradient technique. The identification of structures considered as fractal is in part linked to the scaling characteristics that have to follow power laws. The goodness of fit of the linear regression in the log-log plot is essential. The actual values should lie as close as possible to a straight regression line, and the slope gives an estimate of the fractal dimension. This condition is fulfilled when the R^2 values of the regression is close to 1. Moreover, the histograms of the scattered residuals around this line should follow a normal distribution [58].

Various patterns of variegated SCM pigmentation are observed both in men and women. The interpretation of power spectrum images is generally rather difficult, except when the images have particular sizes or orientations. If the pattern is not really periodic or if it is noisy, the main frequency is hidden by a halo that is denser as the structure becomes more complex [57].

No gender-related differences have been disclosed so far using the parameter of SCM morphometry and fractal analysis.

6. Peritumoral Mosaic Subclinical Melanoderma

Skin photoprotection depends in part on the uppermost keratinocytes with the melanin pigment producing melanocytes present in the basal layer of keratinocytes. Melanin is synthesized in the melanocytes and helps protecting the skin from the deleterious effects of ultraviolet light (UVL) radiations by several mechanisms. Accordingly, patients with impaired production of melanin suffer from a higher incidence of skin cancers. The relationship between the total melanin content, the eumelanin : pheomelanin ratio, and the activity of the key melanogenic enzyme tyrosinase is complex. Mature melanosomes filled with melanin pigment are transported from the melanocyte cell body into the dendrites, to be transferred to the keratinocytes, where they localize to the uppermost perinuclear area.

The mosaic subclinical melanoderma (MSM), also called faint mosaic melanoderma (FMM), is a physiologic pattern of heterogeneous distribution of melanin inside the epidermis. It is influenced by repeat photoexposures since early adulthood [59, 60]. Indeed, the impact of UVL on the epidermis induces an increased production of melanin by melanocytes and its transfer to neighbour keratinocytes in each epidermal melanin unit (EMU). Such EMU activation is responsible for the presentation of MSM, particularly evident in Caucasian skin. A previous study showed that the median value of MSM extent was significantly higher in men with SCM than in women with the same neoplasm [29].

UVL exposure undoubtedly plays a role in the development of SCM, but its involvement is not as clear-cut as for other skin cancers such as squamous cell carcinoma. More risks appear to be associated with intermittent/recreational than with occupational/continuous sun exposure. However, the emphasis on "sun burning" as a cause, rather than a risk factor, is likely misplaced. Thus, for the development of SCM, the most risky sun-related activity for adults is currently thought to be the fortnight's holiday with intense sunlight exposure. The heterogeneity in MSM influences the global photoreactivity of the skin. Although melanin-enriched MSM spots protect locally epidermal cells from UVL, the foci with lower melanin content are much less protected.

7. Conclusion

SCM development is under the influence of genetic factors, age, gender, and environmental influences. The present review was focused on the increasing incidence of SCM developed in women during their childbearing age. Many clinical aspects of the neoplasm are presently reported indistinguishable between genders. By contrast, the peritumoral skin exhibits some gender differences in the extent of physiologic MSM which appears less developed in women than in men. This would suggest that the field effect of prominent sun exposures is not primarily involved in the increasing incidence of SCM in young Caucasian women. These neoplasms generally correspond to thin SCM with a restricted germinative compartment corresponding to a neoplasm exhibiting a slow growth pattern.

The acquired discrete uneven skin pigmentation forming the MSM patterns is a hallmark of photoaging. Once delivered by melanocytes to keratinocytes, melanin acts in part as a UVL-filter. However, according to individual parameters including the phototype, age, and previous cumulative UVL exposures, skin presents distinct FMM appearances. The MSM pattern in men with CMM appeared more heterogeneous with a majority of them showing a large MSM extent.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

A Clinical and Pathological Overview of Vulvar Condyloma Acuminatum, Intraepithelial Neoplasia, and Squamous Cell Carcinoma

Boris Léonard,[1] Frederic Kridelka,[2] Katty Delbecque,[1] Frederic Goffin,[2] Stéphanie Demoulin,[3] Jean Doyen,[2] and Philippe Delvenne[1]

[1] *Department of Pathology, University Hospital of Liège, 4000 Liège, Belgium*
[2] *Department of Gynecology, University Hospital of Liège, 4000 Liège, Belgium*
[3] *Laboratory of Experimental Pathology, GIGA-Cancer, University of Liège, 4000 Liège, Belgium*

Correspondence should be addressed to Philippe Delvenne; p.delvenne@ulg.ac.be

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Condyloma acuminatum, intraepithelial neoplasia, and squamous cell carcinoma are three relatively frequent vulvar lesions. Condyloma acuminatum is induced by low risk genotypes of human papillomavirus (HPV). Vulvar intraepithelial neoplasia (VIN) and squamous cell carcinoma have different etiopathogenic pathways and are related or not with high risk HPV types. The goal of this paper is to review the main pathological and clinical features of these lesions. A special attention has been paid also to epidemiological data, pathological classification, and clinical implications of these diseases.

1. Introduction

Vulvar human papillomavirus (HPV) infection is responsible for the development of benign tumors (condylomata acuminata), of one type of preneoplastic lesions, and of certain types of squamous cell carcinoma (SCC) [1]. Condylomata acuminate are vulvar exophytic benign tumors which result usually (90%) from HPV types 6 and 11 (several other HPV types can be involved) [2]. Up to 83% of women with external genital warts or a history of external genital warts have a concomitant cervical HPV infection [3].

Similarly to the cervix, most of the vulvar (pre)neoplastic lesions are induced by HPV infection (most commonly HPV 16), except for the "differentiated" (simplex) type of VIN.

This allows to distinguish two types of VIN: (1) the usual VIN (uVIN)/classic VIN (WHO terminology) which is related to HPV infection; (2) the differentiated/simplex type (dVIN), non-HPV-related, but associated with vulvar dermatoses, especially the lichen sclerosus [4, 5].

The distinction is also applicable for SCC with "HPV-related SCC," associated with uVIN and "non-HPV-related vulvar SCC," often associated with dVIN and lichen sclerosus [1, 4, 6].

The incidence of HPV-associated VIN, unlike that of vulvar carcinomas, has been increasing over the past 20 years, especially in women of reproductive age, with the highest frequency reported in women of 20–35 years old [7–9]. dVIN type and non-HPV-related vulvar SCC occur commonly in elderly women [9].

Approximately 95% of malignant tumors of the vulva are SCC. They represent 6.38% of all gynaecologic cancers in Belgium (Belgian Cancer Registry 2011). The American Cancer Society reports over 3,400 new cases of vulvar SCC in the USA annually [10].

The incidence of both types (HPV and non-HPV associated) of vulvar SCC increases with age [11, 12]. The reported incidence rates are 1 : 100,000 in younger women and 20 in 100,000 in the elderly [13]. The mean age at presentation is 60–74 years [7, 14]. Vulvar carcinoma may infrequently occur in younger women and adolescents [15, 16]. The peak incidence of vulvar cancer in Belgium (Belgian Cancer Registry 2011) is observed in people over the age of 85.

2. Condyloma Acuminatum ("Genital Warts")

2.1. Clinical Features. Condyloma acuminatum (CA) or venereal/genital warts refer to benign proliferative epidermal or mucosal lesions attributed mostly to HPV type 6 or 11, but coinfections with high-risk HPV types are frequent. More than 100 types of HPV have been identified, of which 40 can infect the genital areas. HPV are highly specific viruses showing both species and regional specificity. They represent the most common sexually-transmitted disease (STD) and are highly contagious. The prevalence of CA peaks in the early sexually active years, with two-thirds of the respective sexual partners complaining of warts. The median time between infection and development of lesions is about 5-6 months among women. Up to 20% of people with genital warts will present other STDs.

The following risk factors have been described, including smoking, hormonal contraceptives, multiple sexual partners, and early coital age. Patients who develop CA complain of painless bumps and, less frequently, of pruritus, discharge, or bleeding. Lesions are commonly multiple (multicentric) and multifocal, also affecting the perianal, vaginal, and cervical regions, but oral and laryngeal regions may also be involved. Latent illness may become active, particularly with pregnancy and immunosuppression. Lesions may regress spontaneously, remain stable, or progress in size and/or number.

CA are soft, raised masses, with smooth, verrucous, or lobulated aspects that may appear as pearly, filiform, fungating, or plaque-like eruptions. The surface commonly shows finger-like projections, generally nonpigmented. They mainly occur in the moist areas of the labia minora and vaginal opening, but virtually, all genital regions may be affected (fourchette, labia minora/majora, pubis, clitoris, urethral meatus, perineum, perianal region, anal canal, introitus, vagina, and ectocervix). Therefore, minutious colposcopic examination, using acetic acid 2–5%, is of crucial importance to detect potentially multiple involved sites.

CA are perceived as disfiguring, they impact sexual lifestyle, causing anxiety, guilt, and loss of self-esteem and creating concerns about cancer risk.

The most common treatments are painful and nonspecific, addressing the clinically evident lesions rather than the viral cause. Various modalities include office-based treatment (cryotherapy, electrocautery, laser, and/or surgery) or home-based treatment (chemotoxic agents or immunomodulatory therapy). First episode patients should be STD screened. Management should include partner notification.

2.2. Etiopathogenesis. The initial site of infection is thought to be either basal cells of the immature squamous epithelium that HPV reaches presumably through defects in the epithelium. Once HPV enters the cells, two distinct biological sequences are possible. The first form is a "nonproductive or latent infection" in which HPV DNA persists in the basal cells without virus replication. Latent infections do not show morphologic alterations and can only be identified using molecular methods.

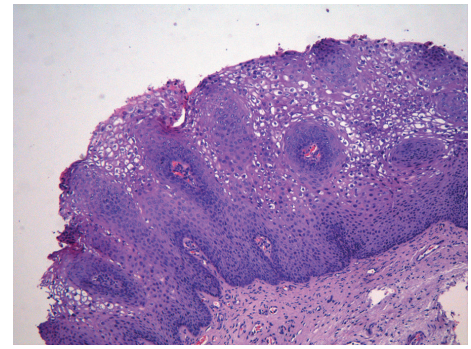

Figure 1: Vulvar condyloma acuminatum with acanthotic squamous epithelium and prominent koilocytic changes.

The second form of HPV infection is a "productive infection." Viral DNA replication in the intermediate and superficial cell layers of the squamous epithelium occurs independently of host chromosomal DNA synthesis. This allows large amounts of intact virions to be formed, leading to typical morphological aspects such as "koilocytic changes."

Molecular biologic methods have identified HPV-6 as the most common HPV type in CA. HPV-11 has been found in approximately one fourth of genital warts. These two HPV types are responsible for over 90% of CA [17, 18].

2.3. Pathologic Features

2.3.1. Gross Findings. The lesions are typically exophytic and may range from discrete papillary excrescences to extensive and coalescent "cauliflower-like" masses [4].

2.3.2. Microscopic Findings. CA shows a striking papillary architecture. Papillae of different sizes and shapes are lined by acanthotic squamous epithelium and have a fibrovascular stroma, often containing scattered chronic inflammatory cells. Hyperkeratosis, parakeratosis, hypergranulosis, and basal cell hyperplasia are seen. Koilocytic changes (rigid perinuclear halos, binucleated nuclei, and slightly enlarged nuclei with irregular contours and coarse chromatin), sometimes focal, are present in the most superficial layers of the squamous epidermis. Mitotic figures are observed in the lower third of the epidermis (Figure 1) [1, 4].

2.3.3. Ancillary Studies. The proliferation index by immunostaining (Ki67/MIB-1) in the upper third of the epithelium is considered as an adjunct test to confirm the diagnosis of CA, especially in lesions without evident koilocytic changes.

The presence of Ki67/MIB-1 immunostaining has been further correlated with the detection of HPV DNA by polymerase chain reaction (PCR).

In situ hybridization for HPV can also be performed on paraffin-fixed tissue sections to confirm the presence of HPV-DNA in CA. However, this test may lack sensitivity [4].

2.4. Treatment Options

2.4.1. Home Therapy

(i) Podophyllotoxin (0.15% cream or 0.5% solution) is an antimitotic and cytotoxic molecule that results in necrosis of genital CA. Each course of podophyllotoxin treatment comprises self-application twice daily for 3 days, followed by four rest days. However, vulvar and anal warts are more feasibly and efficiently treated with clearance rates of 45–83% after use of 0.5% podophyllotoxin solution for 3–6 weeks. Transient and acceptable burning, tenderness, erythema, and/or erosions for a few days when the warts necrotize are described. Recurrence rates of 6–100% have been reported with podophyllotoxin between 8 and 21 weeks after clearance [19–21]. Podophyllotoxin is contraindicated during pregnancy, and women of childbearing age must use contraception.

(ii) Imiquimod 5% topical cream (Aldara) induces interferon production and is a cell-mediated immune response modifier. It is applied to the warts three times a week at bedtime. Treatment continues until wart clearance, or for a maximum of 16 weeks. It presents minimal systemic absorption but causes erythema, irritation, ulceration, and pain at application site. In clinical studies, clearance has been reported in 35–68% of patients with treatment courses up to 16 weeks. Erythema is often seen as a side effect with imiquimod therapy and sometimes appears to precede clinical resolution. Occasionally, severe inflammation is seen necessitating discontinuation of therapy. Relatively low recurrence rates (6–26%) after successful clearance have been reported [13, 22, 23].

(iii) 5-Fluorouracil: no longer recommended for routine use, it has antimetabolic and/or antineoplastic and immunostimulative activity.

(iv) Sinecatechins ointment: Sinecatechins is available as a 10% ointment in Europe and a 15% ointment in the US. It consists in a preparation of green tea catechins (sinecatechins). Evidence suggests that the mechanism of action is made through antiproliferative mechanisms. The ointment is applied three times a day until complete clearance, or for up to 4 months. It cannot be used internally or during pregnancy. Clearance rates of 47–59% over 12–16 weeks were reported. Local side effects occur as frequently as with other topical therapies. They are generally graded as mild and typically include redness, burning, itching, and pain at the site of application. In those clearing, low recurrence rates of 7–11% were observed over 12 weeks follow-up [24–26].

2.4.2. Office Therapy

(i) Trichloroacetic acid (TCA) solution (80–90%) is used directly to the wart surface, weekly. It rapidly penetrates and cauterizes skin, keratin, and other tissues. Although caustic, this treatment causes less local irritation and systemic toxicity and has low cost. Response rates of 56–81% have been reported, with recurrence rates of 36% [27]. TCA can be used safely during pregnancy.

(ii) Cryotherapy may be performed weekly using an open spray or cotton-tipped applicator for 10–20 seconds and repeated as needed. A freeze-thaw-freeze technique is applied to each lesion at each session. Application techniques are difficult to standardize and there may be significant intraoperator differences. Cryotherapy presents the advantages of being simple, inexpensive, rarely causes scarring or depigmentation, and is safe during pregnancy. Clinical studies have reported clearance rates in the range of 44–75%, and recurrence rates of 21–42% one to three months after clearance [27–29].

(iii) Surgical treatments have the highest primary clearance rates with initial cure rates up to 60–90%. They include electrosurgery, curettage, scissors excision, and laser therapy. Surgery may be used as primary therapy, and the majority of patients can be treated under local anaesthesia. When performed carefully, simple surgical approach leaves highly satisfactory cosmetic results. Clearance rates of 94–100% and 89–100% have been reported for electrosurgery and scissors excision, respectively, with recurrence rates of 19–29%. Formal surgery, under anaesthesia, is convenient for the removal of bulky warts, extensive warts, and anal/intra-anal warts.

Many patients either fail to respond to treatment or recur after adequate response resulting in patient dissatisfaction. Global recurrence rates exceed 50% after 1 year due to repeated infection from sexual contact, persistence of virus in surrounding skin, hair follicles, sites not adequately reached by the intervention used, or immunosuppression [27, 30–32].

2.5. Primary Prevention by Prophylactic Vaccine (Quadrivalent Gardasil). A quadrivalent HPV vaccine is available for prevention of HPV-associated dysplasia and neoplasia including cervical cancer, precancerous genital lesions, and genital warts associated with HPV types 6, 11, 16, and 18. Vaccine efficacy is mediated by humoral immune responses following immunization. It is indicated for prevention of CA caused by HPV types 6 and 11 in boys, men, girls, and women aged 9–26 years. The very high efficacy of the quadrivalent HPV vaccine against HPV6/11 disease was reported in multiple randomised, controlled trials. There is now accumulating evidence that population-based quadrivalent HPV vaccination can result in dramatic declines in genital warts incidence and reduction in HPV6/11 burden [33–35].

3. Vulvar Intraepithelial Neoplasia

The term "vulvar intraepithelial neoplasia" (VIN) was endorsed by the International Society for the Study of Vulvar

Disease (ISSVD) in 1986 to describe intraepithelial neoplastic proliferations of the vulvar epidermis [4]. Previously, other terms had been used to describe histologically similar lesions: "Bowen's disease," "erythroplasia of Queyrat," "bowenoid papulosis," and "bowenoid dysplasia" [4]. In 2004, the terminology for squamous VIN was reviewed by the ISSVD that classified VIN in two groups: usual type (uVIN) and differentiated type (dVIN). uVIN type is predominant while dVIN accounts for a small proportion (<2–5%) of all VIN lesions [36, 37]. Both types of VIN have the potential to progress to vulvar cancer.

3.1. Clinical Features. Usual-type VIN (warty, basaloid, or mixed) occurs in young women. The incidence peaks at 45–49 years old, and has increased in recent years and has nearly doubled in the last decades, especially in young women. It is causally related to HPV infection. Other risk factors include smoking and immunosuppression. The lesions are frequently multifocal and have the potential to progress to invasive carcinoma.

Differentiated VIN affects older women, tends to be unifocal, and unicentric. It is not associated with HPV infection, but it is associated with vulvar dermatosis, mainly lichen sclerosus. It also has the potential to progress to invasive carcinoma. VIN is clinically important because the rate of progression to invasive SCC is reported to be as high as 80%, if left untreated [38].

The disease is asymptomatic in about 50% of cases. When symptomatic, the main complaints include itching, pruritus, pain, and dyspareunia. The appearance is variable from unique to multifocal lesions, flat, raised, or eroded, white, grey, red, or brown. After visual or colposcopic examination, such lesions should be biopsied for histological examination. A complete gynaecologic examination is of paramount importance to exclude any multicentric lesions that may affect the cervix, the vagina, and the anal region. The diagnosis of VIN can be subtle. To avoid delay, the physician must exercise a high degree of suspicion. Vulvar biopsy should be used liberally.

3.2. Etiopathogenesis. A part of vulvar carcinogenesis (for uVIN and HPV-related vulvar SCC) is superposable to cervical carcinogenesis. The viral oncoproteins E6 and E7 play a key role in the (pre)malignant transformation. After viral DNA integration in human host-cells, these viral oncoproteins E6 and E7 are overexpressed. Then, E6 degrades the tumor suppressor protein p53, therefore inducing the absence of cell cycle arrest [39]. Moreover, E7 induces an inactive retinoblastoma tumor suppressor gene product, resulting in hyperproliferation of host cells, with overexpression of the cell cycle-related marker p16 [39].

Based on their associations with cervical and anogenital cancers, a nonexhaustive number of anogenital HPVs (HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66,...) have been classified by the International Agency for Research on Cancer (IARC) as oncogenic [40].

Among women infected with high-risk types of HPV, other factors such as smoking, immunosuppression, and long-term use of oral contraceptives can result in a doubling or tripling of risk for HSIL ("high grade squamous intraepithelial lesion") and invasive cancer [41].

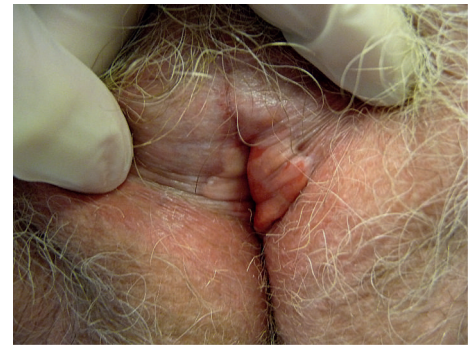

Figure 2: Differentiated VIN developed on sclerous lichen: white and surelevated nodules.

The HPV-independent pathway of vulvar SCC and dVIN is not well known. Genetic mutations in TP53 [42, 43] or PTEN [44] and epigenetic alterations such as hypermethylation of the MGMT, RASSF2A, or TSP1 gene promoters [45] have frequently been detected in dVIN and vulvar SCC, suggesting that the alteration of these genes contributes to vulvar carcinogenesis.

3.3. Pathologic Findings

3.3.1. Gross Findings. Typical low-grade VIN appears as pale areas, whereas high-grade VIN lesions appear as white or erythematous papules or macules that frequently coalesce or show a verrucous growth. Approximately 10–15% of the lesions are hyperpigmented.

Differentiated/simplex VIN can be seen as focal discoloration, ill-defined white plaques, or discrete elevated nodules but they are typically less bulky than uVIN lesions [4]. Approximately two thirds of VIN lesions are multifocal (Figure 2) [1].

3.3.2. Microscopic Findings

(1) Usual/Classic VIN (uVIN). uVIN shows morphological characteristics similar to all HPV-associated intraepithelial lesions such as cervical intraepithelial neoplasia (CIN), anal intraepithelial neoplasia (AIN), vaginal intraepithelial neoplasia (VaIN), and penile intraepithelial neoplasia [46].

These preneoplastic lesions are characterized by epithelial thickening and surface hyperkeratosis and/or parakeratosis. Dysplastic squamous cells with scant cytoplasm and hyperchromatic nuclei are accompanied by dyskeratotic cells with eosinophilic cytoplasm. Nuclear pleomorphism and hyperchromasia are present. However, nucleoli are uncommon. Loss of cell maturation and increased mitotic activity, including abnormal mitotic figures, are also seen [1, 4, 5].

uVIN involves the skin appendages in more than 50% of the cases studied [1]. uVIN is divided into warty ("condylomatous") and basaloid types, essentially based on the architecture and appearance of the intraepithelial lesions.

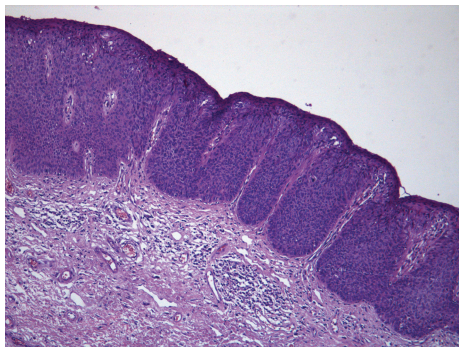

Figure 3: uVIN 3, basaloid type composed of a homogeneous population of dysplastic parabasal type cells on nearly whole thickness of the epidermis.

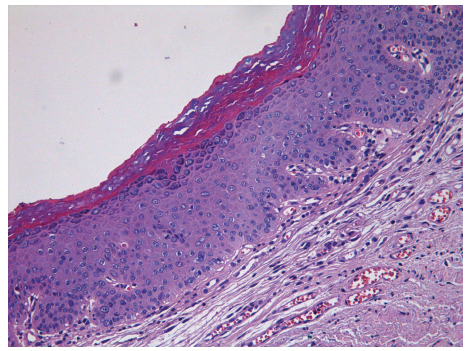

Figure 4: Differentiated VIN: atypical keratinocytes (with large vesicular nuclei with macronucleoli), present in the basal as well as mid layers of the epithelium. No koilocytic changes are identified.

The warty type shows a striking papillary pattern, acanthosis, with cytological signs of viral infection (koilocytic changes, multinucleation, and coarse granules) [1, 4].

The basaloid type presents a flat surface and is composed of a homogeneous population of small atypical parabasal type cells on nearly whole thickness of the epidermis. The epithelium lacks cellular maturation and koilocytic changes which are rarely seen [1, 4]. Sometimes, these two types are present within the same lesion [1, 4].

A rare variant is "pagetoid VIN" where atypical squamous cells present a pale cytoplasm and are isolated or grouped in small clusters [47, 48].

Based on the level of involvement of the thickness of the epithelium by the dysplastic cells, uVIN were graded in 3 grades (WHO terminology [5]):

(i) low-grade (VIN 1) if the dysplastic cells involve the lower third of the epithelium;

(ii) moderate grade (VIN 2) when the dysplastic cells are present in the lower two-thirds of the epithelium;

(iii) high-grade (VIN 3) if there is full-thickness involvement of the epithelium by the dysplastic cells. VIN 3 is synonymous with carcinoma in situ.

It is interesting to note that VIN 2 and VIN 3 confer the same risk and rate of progression to invasive carcinoma if untreated (Figure 3) [4].

The International Society for the Study of Vulvovaginal disease (ISSVD) has proposed that VIN should not be graded but described as high-grade VIN lesions only (VIN 2 or VIN 3). The ISSVD has also recommended that the term low-grade VIN (VIN 1, or mild dysplasia) should not be used anymore and that such lesions should be classified as flat condyloma acuminatum, or given an appropriate descriptive term [49].

(2) Differentiated VIN (dVIN). Differentiated (simplex) VIN is classified as high-grade VIN (thus VIN 3) due to its associated high risk to progress into invasive SCC [1, 4].

It shows epidermal hyperplasia with associated parakeratosis, with elongated and branched rete ridges. An important feature is the finding of squamous cells with abundant bright eosinophilic cytoplasm and typically prominent intercellular bridges. These keratinocytes, present in the basal as well as mid-layers of the epithelium, also show marked cytologic abnormalities such as large vesicular nuclei with macronucleoli.

Mitotic activity is more frequent at the base of the epidermis. No koilocytic changes are identified (Figure 4) [1, 4].

This lesion is frequently associated with lichen sclerosus and other cutaneous inflammation such as lichen simplex chronicus [42, 47].

3.3.3. Ancillary Studies. p16, a surrogate marker of HPV, can be used to detect HPV infection. Diffuse and intense nuclear and cytoplasmic staining typically correlates with high-risk HPV infection. Focal and weak positivity is nonspecific. p16 immunostaining is characteristically negative in the epidermis of dVIN [50, 51].

Detection of increased proliferative activity in the upper layers of the epithelium using Ki67/MIB-1 staining has shown to be well correlated with the presence of HPV DNA by molecular analysis. Ki67/MIB-1 can help for distinction between dVIN and normal vulvar epidermis [52]. HPV in situ hybridization can also be used and is more specific than MIB-1 staining. However, this test suffers from low sensitivity [4].

PCR analysis of VIN 1 lesions has shown a mixture of low- and high-risk HPV (geno)types, whereas VIN 2 and VIN 3 are generally associated with high-risk HPV, most commonly HPV 16 and 18. Molecular studies have failed to demonstrate HPV DNA in dVIN. To identify dVIN, p53 staining can be used. 90% of dVIN show a high p53 positivity in the basal layer with suprabasal extension [42, 47].

3.4. Treatment Options. Due to the natural history of VIN, all VIN lesions should receive treatment. There is little consensus regarding the optimal method of management.

(i) The mainstay of management for VIN has been the surgical excision. One important advantage of surgical excision is that a complete histologic assessment is performed to exclude or define the presence of

occult invasive carcinoma. The goal of the surgery is to obtain a 5-mm disease-free margin to control symptoms and to avoid malignant transformation. Results are initially good, but the recurrence rate is in between 30% to 50% [53]. Large and/or iterative ablations can lead to severe anatomical and functional sequellae, which is particularly distressing in young women. Because of this, and particularly because of the increase in younger women affected, great interest has been paid to nonsurgical treatment of VIN.

(ii) Laser ablative therapy is an alternative to excision. The disadvantage of ablative therapy is that a necrotic ulcer on the vulva may result and wound healing may be slow. Complete healing may take several weeks. Pain, which is severe in some patients, is the main complication with laser therapy. Bleeding and infection have also been reported. The cosmetic results appear to be excellent. Laser therapy is an acceptable treatment modality, if invasive carcinoma has been ruled out. Many consider laser therapy the treatment of choice in the management of VIN, particularly for those who have multifocal disease. In the review article of the 253 patients treated with laser, 23% recurred [53].

(iii) Since most uVIN lesions are associated with high risk-HPV types 16–18 infection, it has been hypothesized that the high recurrence rate following excision or ablative therapies is due to failure to remove the reservoir of HPV present in adjacent vulvar skin. Imiquimod is a low molecular weight imidazoquinoline acting as an immune response modifier, which could have the ability to generate HPV-specific cell-mediated immunity and potentially induce a regression of VIN lesions. Several studies have demonstrated that imiquimod is effective and safe for the treatment of VIN. Imiquimod 5% cream has been approved for the treatment of external anogenital warts and has shown safety and efficacy for different dermatological conditions such as external anogenital warts, superficial basal cell carcinoma, actinic keratosis, and extra genital Bowen's disease. In recent years, randomized control trials have shown that the application of 5% imiquimod is effective in the treatment of high-grade VIN [54]. The first studies had a short term follow-up, so presenting important bias because of the high risk of recurrence even after several years from the primary treatment. Recently, Terlou et al. published a report with seven-year median follow-up showing that in case of complete response, imiquimod is effective in the long-term [55]. However, all the investigations compared the patients treated with imiquimod to a control group treated with placebo, and only few authors analyzed data about the main outcomes in women treated with imiquimod and in women treated with different modalities. Imiquimod seems therefore to offer two important benefits: the avoidance of surgery and a lower recurrence rate for complete responders.

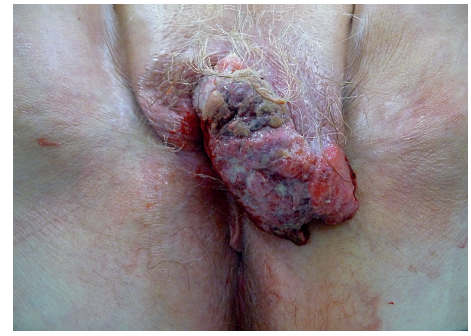

Figure 5: Exophytic and ulcerated squamous cell carcinoma.

The risk factors for VIN recurrence after treatment are smoking, large lesions sizes, surgical specimen with positive margins. Because of the high risk of recurrence and risk of progression to invasive carcinoma, long-term follow-up is mandatory. The ACOG recommends an after therapy visit at 6 and 12 months, and then annually.

3.5. Primary Prevention. Recent randomized controlled trials have demonstrated that sustained protection from VIN can be offered with a prophylactic HPV vaccine. Immunization with HPV vaccination (bi or quadrivalent vaccine) has the potential to prevent about 70% of the VIN.

For example, the quadrivalent vaccine against HPV 6, 11, 16, and 18 was shown to be 97% effective in preventing VIN 2-3 in a population that was naive to these viruses at the time of first vaccination and 100% effective in those who remained unexposed through the completion of the vaccine regimen [56].

It seems that vaccinating HPV-naive women is efficacious, and it would be preferable to vaccinate women before they become sexually active.

4. Invasive Squamous Cell Carcinoma

4.1. Pathologic Features

4.1.1. Gross Findings. SCC may appear as an exophytic or an endophytic ulcerated lesion. The labia majora and minora are preferentially involved. The majority of vulvar SCC are solitary. However, multifocal tumors are seen in 10% of cases (Figure 5) [4]. Clinical symptoms are usually related to the ulceration of the lesion. There is in average 12 to 18 months delay between initial symptoms and definitive histological diagnosis due to prescription of corticoid or antifungal topical therapy without detailed examination of the genitalia.

4.1.2. Microscopic Findings. The current World Health Organization (WHO 2003) classification of vulvar tumors describes several variants of invasive squamous carcinoma [5]:

(a) Keratinizing Squamous Cell Carcinoma NOS (Not Otherwise Specified). This is the most common histologic subtype of

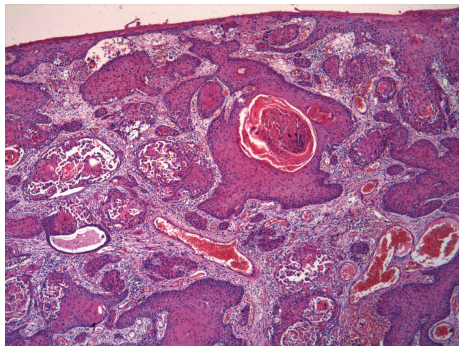

Figure 6: Keratinizing squamous cell carcinoma: infiltrative neoplastic cells are mature with abundant eosinophilic cytoplasm and show keratin pearls.

SCC. Neoplastic cells are mature with abundant eosinophilic cytoplasm and show keratin pearl. The nuclei are enlarged with prominent nucleoli and features readily identified in most cases include considerable nuclear atypia and mitotic activity (Figure 6) [1, 4, 5]. Previously, keratinizing neoplasms are considered as non-HPV-associated tumors. The presence of dVIN and/or lichen sclerosus in adjacent skin was an evidence of an HPV-independent implication [50].

Recently, typing by "polymerase chain reaction" showed a significant number of discrepancies: prevalence of HPV in keratinizing SCC is observed up to 49.1% [57].

(b) Nonkeratinizing Squamous Cell Carcinoma. The cells in this subtype of invasive SCC show minimal evidence of keratinization with scattered keratinized cells but lacks keratin pearl. Keratin pearl formation is not observed [1, 4, 5]. Prevalence of HPV in nonkeratinizing SCC is 85.7% [57].

(c) Basaloid Carcinoma. This tumor subtype arises in association with high-grade uVIN and comprises irregular nests and cords of "basaloid" cells with scant cytoplasm. The cells are ovoid, relatively uniform in size, and the nuclei show evenly distributed granular chromatin with no evident nucleoli. No keratin pearls are observed [1, 4, 5]. Prevalence of HPV in basaloid SCC is 92.3% [57]. HPV-16 can be detected in approximately 70% of cases [1, 58].

(d) Warty (Condylomatous) Carcinoma. This histologic subtype is architecturally characterized by the presence of multiple papillary projections with fibrovascular cores. The papillae are lined by keratinized squamous epithelium showing koilocytic changes (the most characteristic features). Keratin pearl formation in the invasive nests is often seen [1, 4, 5, 59, 60]. Prevalence of HPV in warty carcinoma is 78.2% [57]. HPV 16 is frequently observed [59, 60].

(e) Verrucous Carcinoma. This highly differentiated variant of SCC is characterized by bulbous pegs of neoplastic cells that appear to push into the underlying stroma. The neoplastic squamous epithelium is hyperplastic and associated with prominent hyper- and parakeratosis. The tumor cells have abundant cytoplasm. Nuclear atypia is minimal. Mitotic figures are rare and koilocytosis is usually absent. Verrucous carcinoma presents no or very little metastatic potential. HPV type 6 has been identified in a number of verrucous carcinoma [4, 5, 61–64], but it is controversial in the literature; a recent retrospective study does not support a causal role of HPV in the development of verrucous carcinoma [65].

(f) Squamous Carcinoma with Tumor Giant Cells. This is a rare and aggressive variant of invasive SCC characterized by the presence of numerous multinucleated tumor giant cells. Large atypical nuclei with prominent nucleoli and brisk mitotic activity are frequent [4, 5].

(g) Keratoacanthoma-Like Carcinoma. This variant has been included in the last WHO classification (2003) of vulvar SCC. It presents an appearance of keratoacanthoma and occurs on hair-bearing skin. Histologically, it is characterized by the presence of a central crater filled with proliferating squamous epithelium and anucleated masses of keratin. Invading nests and cords of squamous epithelium are observed in the dermis. These tumors may regress spontaneously by a poorly understood immune mechanism [4, 5].

There is no grading system unanimously accepted for vulvar SCC. The American Joint Committee on Cancer (AJCC 2010) recommends a four-grade system: well-differentiated (G1), moderately differentiated (G2), poorly differentiated (G3), and undifferentiated (G4) (GX, grade cannot be assessed) [66].

The grading system recommended by the Gynecologic Oncology Group (GOG) is based on the percentage of undifferentiated cells (small cells with scant cytoplasm infiltrating the stroma).

Grade 1 tumors have no undifferentiated cells, Grade 2 tumors contain less than 50% undifferentiated cells, Grade 3 tumors have greater than 50% but less than 100%, and grade 4 is essentially entirely composed of undifferentiated cells. The risk of recurrence has been reported to be higher with increasing grade [67–69].

4.1.3. Ancillary Studies. Immunohistochemical expression of p16 is commonly expressed in vulvar squamous tumors and VIN associated with oncogenic HPV [70, 71]. Indeed, the sensitivity and specificity of p16 immunostaining are close to 100% for detecting HPV-related carcinomas [50, 72]. Therefore, the anti-p16 antibody may be used as a good alternative to PCR [50, 73]. p53 staining is positive in 50–70% of HPV-unrelated vulvar SCC, which contrasts with a negativity of p53 staining in almost HPV-related vulvar SCC [50].

4.2. Treatment Options. Vulvar carcinoma benefits from a surgical staging system based on criteria established by the International Federation of Gynecology and Obstetrics (FIGO) [74]. The latter includes variables related to the primary disease (early or locally advanced stage) and the nodal status (negative versus ipsilateral or bilateral positivity).

Pretreatment assessment includes confirmation biopsy and careful perineal, vulvar, and vaginal examination.

MRI and PET CT are not part of a routine work up and must be prescribed on individual basis.

Early stage carcinoma is best treated by a radical local excision with macroscopic tumor-free margins of 1 cm [75]. The "traditional" radical vulvectomy is no longer systematically applied due to its major deleterious impact on vaginal function. In the same operating time, nodal staging is justified when tumor depth exceeds 1 mm. Bilateral radical inguinofemoral (IF) nodal dissection carries a heavy potential morbidity (lymphocele, lymphoedema) and must therefore be individualized [76]. The sentinel node approach is nowadays considered validated for early stage neoplasms with a greater diameter <2 cm [77]. For early stage disease >2 cm, IF nodal staging must include superficial inguinal and deep femoral node dissection [78]. The procedure may be carried out ipsilaterally in case of labia major lateralised disease. The dissection must be bilateral in case of midline disease (minor labia, periclitoral, periurethral, or perianal) [79].

Locally advanced vulvar carcinoma based on vaginal, urethral, or anal involvement is treated by concomitant chemoradiation associating external beam, brachytherapy implant, and radiosensitizing platinum chemotherapy. In this context, nodal staging may precede the initiation of the radiotherapy [80]. External beam target volume may then be tailored on the basis of the patient's nodal status and limit the radiation induced morbidity in the absence of nodal metastases.

Recommended follow-up includes clinical vulvar, vaginal, and nodal examination on a three to six monthly basis. Indication of routine morphological or metabolic imaging exams must be individualized.

5. Conclusion

The understanding of all the aspects of these three relatively frequent diseases that are "vulvar condyloma acuminatum, vulvar intraepithelial neoplasia, and vulvar SCC" guarantees an optimal care of the patients. A close cooperation between clinicians and pathologists is also essential concerning their accurate diagnosis.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Measurement of Urinary Biomarkers of Parabens, Benzophenone-3, and Phthalates in a Belgian Population

Lucas Dewalque,[1,2] Catherine Pirard,[1,2] and Corinne Charlier[1,2]

[1] *Laboratory of Clinical, Forensic and Environmental Toxicology, University of Liege (ULg), CHU (B35), 4000 Liege, Belgium*
[2] *Center for Interdisciplinary Research on Medicines (CIRM), University of Liege (ULg), CHU (B35), 4000 Liege, Belgium*

Correspondence should be addressed to Lucas Dewalque; lucas.dewalque@ulg.ac.be

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Parabens, benzophenone-3 (BP3), and phthalates are commonly used as antimicrobial conservator, UV-filter, and plasticizer, respectively, and are thought to exhibit endocrine disrupting properties. These endocrine disrupting activities have been recently assumed to lead to cutaneous malignant melanoma. Humans are exposed to these chemicals through different sources such as food, personal care products, or cosmetics. In this study, we measured urinary levels of 4 parabens, BP3, and 7 metabolites of phthalates in samples collected from 261 participants living in and around Liege (Belgium). The analyses were carried out by liquid chromatography tandem mass spectrometry (LC-MS/MS) using isotopic dilution. To the best of our knowledge, this is the first time that the urinary levels of these 3 classes of chemicals are reported for the same general population in Belgium. Most of the parabens, the BP3, and all the phthalate metabolites were detected in 82.8 to 100.0% of the samples. For most of these chemicals, the exposure patterns significantly differ not only between children and adults, but also between males and females, especially with higher concentrations of parabens and phthalate metabolites in female and children subjects, respectively.

1. Introduction

Numerous studies have demonstrated the alarming increase of cutaneous malignant melanomas (CMM) in Caucasian populations these last decades [1–5]. CMM is known to occur mainly in women aged between 15 and 34, although the higher incidence for this specific subpopulation is not well understood [1–3]. While genetic predispositions [4] or environmental factors such as natural or artificial ultraviolet light exposure could induce CMM [5], the exposure to man-made chemicals such as persistent organic pollutants or pesticides was suspected to explain the overall increasing CMM incidence [6–8] but strong evidence is still lacking. Focusing on environmental pollutants, the endocrine disrupting chemicals, which are known to interact with the hormonal homeostasis, are thought to act on estrogen receptor present in melanoma cells [9, 10] or alter HOX genes function which seem to be correlated with tumor progression [11, 12]. Furthermore, some authors recently hypothesized a link between the higher exposure to some endocrine disrupting chemicals, namely, UV-filters and parabens, and the increasing incidence of CMM [10, 13]. In this paper, we tried to assess the human exposure of 3 classes of endocrine disruptors, namely, parabens, benzophenone-3 (BP3), and phthalates. For this purpose, we measured their urinary biomarkers.

Methyl- (MP), ethyl- (EP), n-propyl- (PP), and n-butylparaben (BP), which are some esters of the parahydroxybenzoic acid (PHBA), are widely used alone or in combination as an antimicrobial conservator in personal care products (cosmetics, shampoos, shaving products, lotions, etc.) but also in food, beverages, food packaging, and pharmaceutical preparations [14, 15]. When present in food, the parabens are orally absorbed and rapidly degraded by liver esterases to PHBA, which is rapidly eliminated in urine as unspecific biomarker [14]. After dermal application of personal care products containing parabens, most of them are degraded by some skin esterases and only a small fraction is available to cross the epidermis and reach the systemic circulation. The unchanged parabens are then excreted in urine as glucuronide, glycine, and sulfate conjugates and could be

therefore used as specific biomarkers to assess their exposure [14, 16, 17]. Although they used to be considered as slightly toxic, the parabens have been demonstrated to show *in vitro* and *in vivo* weak estrogenic activity [18–20]. They can also alter the reproductive functions in male rats and mice after *in utero* exposure [21–23]. The human health effects of the paraben exposure at environmental levels are still unknown and their toxicity remains controversial since several studies did not achieve to demonstrate the endocrine disruptor effects [15, 24, 25]. Nevertheless, parabens have been suspected to be involved in melanocytic lesions [13] because, on the one hand, they can interact with the estrogen receptor beta [26, 27] present in melanoma cells and therefore influence the development of the tumors [9], and on the other hand, they can potentiate UV-induced damage in keratinocyte through oxidative stress [28]. It has been shown that women used to be more exposed to parabens because of their more frequent use of personal care products [29, 30]. Moreover a higher incidence of CMM has been demonstrated in women [1–3]. Consequently, the potential involvement of parabens exposure in CMM incidence can be explored, although, until now, the influence of these endocrine disruptors on the physiopathology of melanoma has never been demonstrated.

BP3 used to be added in sunscreens and cosmetics as a UV-filter but was also introduced in plastic surface coatings and polymers as a UV-stabilizer [10, 31, 32]. Following dermal exposure, BP3 is absorbed through the skin [32] and eliminated in the urine mainly as glucuroconjugated species after phase I and phase II metabolism [32, 33]. Since glucuroconjugated forms are excreted in urine in large amount, unchanged BP3 used to be monitored after hydrolysis step as a specific biomarker [32, 33]. BP3 is known to exhibit estrogen agonist properties and androgen antagonist activities [31, 34, 35]. In biomonitoring studies, higher BP3 exposure has been observed in the female population, probably also due to its presence in personal care products [36].

Phthalates are commonly used as plasticizer especially in PVC but also as solubilizing and stabilizing agent in a broad range of other applications. They can be found in various everyday life products like children toys, cosmetics, and perfumes, as well as in building materials such as vinyl flooring, in food packaging, in adhesives, in clothes, or in medical materials and drugs [57]. Since phthalates are not chemically bound to the polymers, they can be released into the environment. Their exposure can therefore occur through various sources, mainly food but also through air dust, water, use of personal care products, or parenteral way for individuals undergoing medical procedures [61]. In some animal toxicity studies, phthalates were shown to influence the endogenous production of several hormones like testosterone, insulin-like factor 3, and follicle-stimulating hormone and thus could be related to functional and structural impairment of male reproduction and development [61]. The human exposure to phthalates has been associated with alteration of sperm quality [62], reduced anogenital distance in infant [63], neurodevelopment disorders [64], and increased waist circumference and insulin resistance [65]. The exposure assessment of phthalates is carried out using biomonitoring approaches consisting in the measurement of their urinary metabolites, which are the corresponding monoesters oxidized or not [61].

This work is the first part of a larger study which will focus on the potential link between melanoma and exposure to endocrine disrupting chemicals. For this purpose, the establishment of some reference values in the Belgian general population is needed. Therefore, in order to determine these levels of background contamination, we measured urinary levels of 4 parabens (methyl-, ethyl-, propyl-, and butylparaben), BP3, and 7 metabolites of phthalates, namely, monoethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), mono-iso-butyl phthalate (MiBP), monobenzyl phthalate (MBzP), mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (5-OH-MEHP), and mono-2-ethyl-5-oxohexyl phthalate (5-oxo-MEHP), in 261 people aged between 1 and 85, living in Liege or the surrounding areas.

2. Material and Methods

2.1. Sample Collection. This study was approved by the Hospital Faculty Ethics Committee of the University of Liege (Belgium). 261 healthy females and males aged from 1 to 85, living in Liege or in the surrounding areas and having no occupational activity related to phthalates, parabens, or BP3, signed free and informed consent. The participants filled in a short questionnaire including data about age, weight, size, smoking habits, and residence localization. For children, the consent and the questionnaire were filled in by the parents or the person in charge. The characteristics of the study population are detailed in Table 1. As summarized in this table, the participants were classified into 3 groups depending on their residence place and based on the Eurostat concept of the rural and urban communities [66]. Therefore these places of residence were defined according to the population density and the total number of inhabitants as densely populated (>500 inhabitants/km^2 and ≥50,000 inhabitants), intermediately populated (between 100 and 500 inhabitants/km^2 and ≥50,000 inhabitants), and sparsely populated (≤100 inhabitants/km^2 and <50,000 inhabitants). Spot urine samples were collected in 100 mL polypropylene containers previously screened for potential contamination of phthalate metabolites, BP3, and parabens. The sample collection was carried out from January to April 2013. Immediately after the collection, samples were aliquoted and frozen at −20°C since the phthalate metabolites were demonstrated to be stable in these conditions for at least one year [67] and parabens and BP3 for 6 months [68].

2.2. Phthalate Metabolites, Parabens, and BP3 Analysis. The optimization and validation of the analytical procedure for the simultaneous determination of the 7 phthalate metabolites, the 4 parabens, and the BP3 have been previously described [69]. Briefly, after the addition of internal standard and sodium acetate buffer to 3 mL of previously centrifuged urine, the samples were hydrolyzed overnight at 37°C using *Helix pomatia* glucuronidase. Then samples were acidified

using 200 μL of formic acid, centrifuged again, and the supernatants were loaded on the SPE Bond Elut Certify LRC cartridges which had previously been conditioned. The cartridges were then washed with acetic acid and eluted twice with acetonitrile. The eluate was then evaporated until dryness under a nitrogen gentle flow at 40°C and reconstituted in 70 μL of a 70 : 30 (v:v) acidified water-acetonitrile solution. Finally, the extracts were centrifuged one last time prior to analysis, performed by UHPLC-MS/MS in positive electrospray mode (ESI) for BP3 and negative ESI for parabens and phthalate metabolites. The separation was carried out using a Kinetex Phenyl-Hexyl column 100 × 2.1 mm, 1.7 μm with acidified water and acetonitrile as mobile phases. The LC gradient, the specific parameters of the mass spectrometry, and the characteristics of the MS/MS transitions have been detailed elsewhere [69].

2.3. Urinary Creatinine Determination. The creatinine measurements were carried out using the automate ARCHITECT ci 4100 (Abbott, Illinois, USA) and the Abbott reagents and calibration kits. The analysis method was based on enzymatic chain reactions and absorbance measurements.

2.4. Determination of Unknown Samples. The determination of unknown samples was carried out using calibration curves ranging from 0.5 to 200 μg/L (except MP and BP3 from 2 to 800 μg/L) in synthetic urine. When the concentration measured was above the highest calibration point, the analysis was rerun on diluted samples with synthetic urine. Each sequence of unknown samples included a procedural blank (constituted of synthetic urine) and two level home-made quality controls (10 and 100 μg/L for each compound except BP3 and MP, 40, and 400 μg/L) [69]. Moreover, our lab participated and successfully passed the German External Quality Assessment Scheme (G-EQUAS) 2013 program, in which human urine control materials 51-9A and 51-9B were analyzed for MnBP, MiBP, MBzP, MEHP, 5-OH-MEHP, and 5-oxo-MEHP [69].

2.5. Statistical Analysis. The values below our limits of detection (LOD) were treated as LOD/2 in the statistical analyses [37, 43, 47, 70]. Kruskal-Wallis test, Mann-Whitney *U* test and Spearman's rank correlation were performed using GraphPad Prism 5.0 software (GraphPad Software, CA, USA) to compare biomarker levels measured according to the age group and the gender and to highlight associations. Microsoft Office Excel 2003 (Microsoft Corporation, Washington, USA) was used to determine percentiles and geometric means (GM). Significance limit was set at 0.05.

3. Results and Discussion

Creatinine adjustment is commonly used to take into account the volume dilution in environmental biomonitoring studies. Actually several studies suggested that creatinine adjustment could induce bias when comparing different populations such as ethnical groups, pregnant women, neonatal, children, or the elderly for whom creatinine excretion could be impacted by physiological factor not directly related to their environmental exposure, for instance, renal function, muscle mass, sex, ethnicity, food consumption, and age [57, 59, 71–75]. For these reasons, creatinine adjustment is more and more discouraged in biomonitoring studies [76]. Therefore, the results are presented here in both μg/L and μg/g creatinine, but all statistical analyses and discussions were performed on unadjusted concentrations. For each of biomarkers measured, unadjusted urinary levels were highly or very highly correlated with their respective creatinine adjusted concentrations ($r = 0.75$–0.97 $P < 0.001$) excepted for MEHP for which correlation was moderate ($r = 0.56$ $P < 0.001$). The Mann-Whitney *U* test did not highlight any significant difference in biomarkers levels according to the place of residence. No statistics were performed on the influence of smoking habits because of the very small proportion of smokers in the studied population (Table 1).

Table 1: Demographic details on the studied population.

	Men	Women
N (%)	123 (47.1%)	138 (52.9%)
1 to 6 years	12	11
1 to 3 years	*3*	*6*
4 to 6 years	*9*	*5*
7 to 11 years	11	14
12 to 19 years	15	15
20 to 39 years	46	53
40 to 59 years	24	26
≥60 years	15	19
Average age (min–max) (years)	31.3 (2–75)	31.9 (1–85)
BMI (kg/m^2)		
BMI < 18.5	23.5%	23.9%
18.5 ≤ BMI < 25	60.9%	46.0%
25 ≤ BMI < 30	11.7%	23.9%
BMI ≥ 30	3.9%	6.2%
Placed residence		
Densely populated	51.3%	51.1%
Intermediately populated	43.5%	40.6%
Sparsely populated	5.2%	8.3%
Smoker		
Yes/no	6.1%/93.9%	5.4%/94.6%

GM, the percentiles (5th, 25th, 50th, 75th, and 95th), the range, and the frequencies of detection are detailed in Table 2 for the 261 participants. All subjects were categorized into six age groups (1–6; 7–11; 12–19; 20–39; 40–59; ≥60 years) including a minimum of 23 participants and homogeneously distributed according to the sex (Table 1). The median biomarker levels were also presented according to the different age groups in Table 3.

3.1. Parabens. MP was detected in all the urine samples at concentrations ranging from 0.3 to 7576 μg/L and at a GM of 19.0 μg/L (Table 2). EP and PP were positively detected in 96.6% and 83.1% of the urine samples, respectively, and their GM levels were 2.1 μg/L and 1.5 μg/L, ranging from <LOD

Table 2: Urinary concentrations of parabens, BP3, and phthalate metabolites (μg/L or μg/g creatinine): geometric means (GM), percentiles, ranges, and positivity rates.

	GM μg/L (μg/g creat.)	Selected percentiles μg/L (μg/g creat.)					Range μg/L (μg/g creat.)	Positive samples (%)[a]	LOD (μg/L)
		5th	25th	50th	75th	95th			
MP									
All (n = 261)	**19.0 (16.9)**	**1.1 (1.0)**	**4.3 (3.6)**	**16.1 (14.3)**	**75.2 (61.1)**	**462.6 (501.7)**	**0.30–7576.0 (0.23–3712.0)**	**100.0**	0.16
Male (n = 123)	10.1 (7.8)	1.0 (1.0)	3.3 (2.8)	7.7 (5.1)	26.9 (16.4)	223.4 (263.5)	0.30–2659.0 (0.23–2227.0)	100.0	
Female (n = 138)	33.5 (33.5)***	1.0 (1.5)	11.0 (9.8)	32.4 (32.1)	115.1 (128.7)	630.6 (612.7)	0.37–7576.0 (0.28–3712.0)	100.0	
EP									
All (n = 261)	**2.1 (1.8)**	**0.1 (0.2)**	**0.6 (0.5)**	**1.7 (1.5)**	**6.5 (5.7)**	**67.7 (53.9)**	**<LOD–887.3 (<LOD–1033.0)**	**96.6**	0.09
Male (n = 123)	1.6 (1.3)	0.1 (0.1)	0.5 (0.4)	1.3 (1.0)	4.7 (3.5)	41.4 (26.4)	<LOD–887.3 (<LOD–592.3)	95.9	
Female (n = 138)	2.6 (2.6)*	0.1 (0.2)	0.8 (0.7)	1.9 (2.1)	9.2 (8.8)	83.1 (90.5)	<LOD–452.8 (<LOD–1033.0)	97.1	
PP									
All (n = 261)	**1.5 (1.3)**	**<LOD (<LOD)**	**0.2 (0.2)**	**1.2 (1.0)**	**9.3 (8.4)**	**78.8 (89.2)**	**<LOD–692.1 (<LOD–415.1)**	**83.1**	0.11
Male (n = 123)	0.6 (0.5)	<LOD (<LOD)	0.1 (0.1)	0.5 (0.4)	2.2 (1.6)	20.2 (15.0)	<LOD–114.6 (<LOD–156.2)	76.4	
Female (n = 138)	3.3 (3.3)***	<LOD (<LOD)	0.8 (0.6)	3.3 (3.5)	15.4 (19.6)	116.5 (215.1)	<LOD–692.1 (<LOD–415.1)	89.1	
BP									
All (n = 261)	**ND (ND)**	**<LOD (<LOD)**	**<LOD (<LOD)**	**<LOD (<LOD)**	**0.9 (0.8)**	**8.0 (8.5)**	**<LOD–80.6 (<LOD–63.5)**	**41.8**	0.30
Male (n = 123)	ND (ND)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	0.3 (0.2)	4.9 (3.8)	<LOD–19.4 (<LOD–12.4)	23.6	
Female (n = 138)	ND (ND)***	<LOD (<LOD)	<LOD (<LOD)	0.5 (0.5)	1.7 (1.7)	11.1 (10.3)	<LOD–80.6 (<LOD–63.5)	58.0	
MEP									
All (n = 261)	**37.6 (33.3)**	**4.9 (5.7)**	**16.0 (14.7)**	**34.3 (30.6)**	**88.5 (68.4)**	**292.1 (251.8)**	**2.23–1904.0 (2.73–860.9)**	**100.0**	0.28
Male (n = 123)	40.2 (31.1)	5.2 (5.2)	15.6 (15.6)	34.3 (34.3)	93.9 (93.9)	731.9 (731.9)	2.65–1904.0 (2.65–1904.0)	100.0	
Female (n = 138)	35.4 (35.4)	4.9 (4.9)	16.5 (16.5)	34.5 (34.5)	85.2 (85.2)	216.9 (216.9)	2.23–578.0 (2.23–578.0)	100.0	
MnBP									
All (n = 261)	**31.3 (27.7)**	**5.7 (7.1)**	**15.5 (14.7)**	**33.3 (26.1)**	**63.1 (53.4)**	**145.9 (112.6)**	**2.00–235.6 (3.63–422.0)**	**100.0**	0.30
Male (n = 123)	33.0 (25.5)	6.7 (6.9)	17.0 (12.1)	34.8 (26.1)	61.1 (46.9)	150.4 (91.1)	3.23–222.7 (3.63–143.1)	100.0	
Female (n = 138)	29.8 (29.9)	5.3 (8.6)	14.9 (15.5)	31.4 (26.1)	63.4 (60.4)	116.5 (126.7)	2.00–235.6 (4.66–422.0)	100.0	
MiBP									
All (n = 261)	**26.2 (23.3)**	**5.9 (6.9)**	**14.3 (12.4)**	**24.3 (20.0)**	**45.9 (38.5)**	**154.4 (147.3)**	**2.04–608.4 (3.09–473.1)**	**100.0**	0.37
Male (n = 123)	24.9 (19.3)	5.7 (5.4)	13.5 (10.5)	24.3 (17.2)	43.4 (29.0)	99.6 (99.7)	2.46–504.9 (3.09–307.2)	100.0	
Female (n = 138)	27.5 (27.5)	6.2 (8.6)	15.0 (14.3)	24.6 (22.4)	48.1 (50.5)	169.5 (174.3)	2.04–608.4 (5.93–473.1)	100.0	
MBzP									
All (n = 261)	**5.5 (4.9)**	**0.9 (1.2)**	**2.9 (2.3)**	**5.5 (4.4)**	**10.3 (9.1)**	**34.9 (28.5)**	**<LOD–126.5 (<LOD–102.0)**	**99.6**	0.19
Male (n = 123)	5.8 (4.5)	1.0 (1.0)	3.0 (3.0)	5.8 (5.8)	11.3 (11.3)	40.5 (40.5)	0.40–126.5 (0.65–102.0)	100.0	
Female (n = 138)	5.2 (5.2)	0.8 (0.8)	2.6 (2.6)	5.3 (5.3)	10.1 (10.1)	34.3 (34.3)	<LOD–84.0 (<LOD–73.0)	99.3	
MEHP									
All (n = 261)	**2.7 (2.4)**	**1.1 (0.7)**	**1.7 (1.5)**	**2.7 (2.3)**	**4.1 (4.0)**	**8.7 (7.8)**	**0.58–20.0 (0.51–46.5)**	**100.0**	0.19
Male (n = 123)	3.0 (2.4)**	1.3 (0.7)	1.9 (1.5)	3.0 (2.1)	4.6 (3.6)	8.7 (6.8)	0.99–13.4 (0.51–9.7)	100.0	
Female (n = 138)	2.5 (2.5)	1.0 (0.7)	1.5 (1.4)	2.2 (2.3)	3.8 (4.2)	8.7 (8.5)	0.58–20.0 (0.56–46.5)	100.0	
5-OH-MEHP									
All (n = 261)	**8.6 (7.6)**	**1.8 (2.0)**	**4.7 (4.3)**	**9.0 (6.8)**	**15.3 (13.4)**	**36.7 (35.1)**	**0.31–113.0 (0.96–263.5)**	**100.0**	0.13
Male (n = 123)	10.1 (7.8)**	2.5 (2.0)	5.6 (4.5)	9.9 (7.3)	19.4 (13.8)	37.3 (33.4)	1.33–59.3 (1.47–41.8)	100.0	
Female (n = 138)	7.4 (7.4)	1.2 (1.8)	3.7 (4.1)	7.9 (6.5)	13.7 (12.2)	37.4 (36.4)	0.31–113.0 (0.96–263.5)	100.0	

Table 2: Continued.

	GM μg/L (μg/g creat.)	Selected percentiles μg/L (μg/g creat.)					Range μg/L (μg/g creat.)	Positive samples (%)[a]	LOD (μg/L)
		5th	25th	50th	75th	95th			
5-oxo-MEHP									
All (n = 261)	**5.8 (5.1)**	**0.9 (1.4)**	**3.1 (2.8)**	**5.9 (4.8)**	**11.2 (8.7)**	**27.4 (23.4)**	**0.29–94.7 (0.67–220.7)**	**100.0**	0.16
Male (n = 123)	6.5 (5.0)	1.4 (1.4)	3.8 (2.7)	6.7 (4.8)	12.2 (9.0)	26.9 (21.9)	0.87–57.4 (0.67–36.8)	100.0	
Female (n = 138)	5.3 (5.3)	0.8 (1.4)	2.9 (2.9)	5.6 (4.9)	10.2 (8.1)	35.2 (26.6)	0.29–94.7 (0.80–220.7)	100.0	
BP3									
All (n = 261)	**1.3 (1.1)**	**<LOD (<LOD)**	**0.4 (0.3)**	**1.3 (1.0)**	**3.7 (3.1)**	**32.2 (30.1)**	**<LOD–662.8 (<LOD–414.2)**	**82.8**	0.20
Male (n = 123)	1.1 (0.8)	<LOD (<LOD)	0.3 (0.2)	0.9 (0.6)	3.1 (2.0)	34.5 (28.8)	<LOD–662.8 (<LOD–414.2)	82.1	
Female (n = 138)	1.4 (1.4)	<LOD (<LOD)	0.4 (0.4)	1.7 (1.3)	3.8 (4.4)	32.7 (33.3)	<LOD–140.0 (<LOD–141.3)	83.3	

[a]samples above LOD (%).
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 3: Paraben, BP3, and phthalate metabolite medians (μg/L) in the different age groups.

Age groups (N)	1–6 (23)	7–11 (25)	12–19 (30)	20–39 (99)	40–59 (50)	≥60 (34)
MP	34.8	9.1	18.0	13.3	25.6	13.0
EP	2.3*	0.7	1.1	1.9*	2.2*	2.6*
PP	2.1	0.8	4.2	1.0	2.3	0.8
BP	0.8*	<LOD	<LOD	<LOD	<LOD	<LOD
BP3	1.8	1.4	3.6**	0.9	1.3	0.5
MEP	33.3	39.2	42.4	27.5	34.5	54.0
MnBP	59.0***	48.4*	40.9*	24.8	30.7	29.4
MiBP	59.5***	64.1***	33.6**	19.3	21.9	14.8
MBzP	10.2*	8.2*	8.4*	4.2	4.1	4.6
MEHP	3.4**	3.0*	3.7*	2.8*	2.2	1.8
5-OH-MEHP	21.7***	14.3**	13.7***	7.3	5.8	6.0
5-oxo-MEHP	17.1***	10.2***	9.5***	4.6	3.8	3.8

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 4: Spearman's rank correlations between urinary phthalate metabolites, parabens, and BP3.

	MEP	MnBP	MiBP	MBzP	MEHP	5-OH-MEHP	5-oxo-MEHP	MP	EP	PP	BP	BP3
MEP	—											
MnBP	0.51***	—										
MiBP	0.32***	0.64***	—									
MBzP	0.44***	0.68***	0.60***	—								
MEHP	0.18***	0.35***	0.34***	0.42***	—							
5-OH-MEHP	0.27***	0.54***	0.56***	0.62***	0.69***	—						
5-oxo-MEHP	0.24***	0.54***	0.59***	0.60***	0.70***	0.96***	—					
MP	0.25***	0.20**	0.19**	0.14*	0.07ns	0.12ns	0.12*	—				
EP	0.35***	0.30***	0.19**	0.15*	0.04ns	0.14*	0.08ns	0.55***	—			
PP	0.25***	0.23***	0.17**	0.11ns	0.09ns	0.08ns	0.07ns	0.79***	0.48***	—		
BP	0.17**	0.35***	0.32***	0.24***	0.16**	0.24***	0.25***	0.51***	0.46***	0.53***	—	
BP3	0.25***	0.35***	0.37***	0.25***	0.15*	0.33***	0.33***	0.27***	0.28***	0.33***	0.37***	—

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns: not significant.

to 887 μg/L and <LOD to 692 μg/L, respectively. Unlike the other parabens, BP showed a poor detection rate (41%) which did not allow us to determine GM and perform statistics and showed globally lower urinary levels (from <LOD to 81 μg/L). Whatever the targeted paraben is, significantly higher levels were observed in the urine of women compared to men ($P = 0.040$–<0.0001). This observation was consistent with the NHANES study on the American general population [29] and was most likely related to the higher use by women of personal care products such as cosmetics which may contain parabens. Moreover, a recent study highlighted the association between fresh application of cosmetics and higher paraben exposure [30]. Focusing on the urinary paraben levels according to the age group (Table 3), EP levels were significantly lower in the age group of 7–11 years, while conversely BP concentrations were statistically higher in young children (1–6 years) compared to teenagers and young adults (12–39 years) and to the older group (≥60 years). If higher EP levels in the adults could be probably explained by more important use of personal care products or pharmaceutical preparations containing EP, the reason why young children seemed to be more exposed to EP and BP was unclear. On the other hand, the levels of the four studied parabens were correlated ($r = 0.46$–0.79 $P < 0.001$) and especially MP and PP ($r = 0.79$ $P < 0.001$) as detailed in Table 4. This suggested potential common sources of exposure for the different parabens known to be used in combination in personal care products, pharmaceutical preparations, or food [15, 16]. Furthermore, MP and PP are reported to be more frequently combined parabens [16] and were also strongly correlated in other biomonitoring studies [29, 30, 37, 39, 41]. Conversely, Shirai et al. [43] did not observe such a significant correlation between parabens in Japanese pregnant women. The apparent inconsistence with the Asian study might be the result of different paraben use in commercial products from one country to another, yielding to different exposure between populations.

Table 5 gathers the urinary paraben results from different national large-scale biomonitoring studies for children and adults. The highest paraben concentrations in children urine were reported in four-year-old Spanish boys [37]. Excluding this Spanish study, the paraben levels found in our Belgian children seemed to be close to those usually measured in other countries except for MP detected, respectively, at higher

Table 5: Paraben and BP3 concentrations—medians (95th percentile) in μg/L—reported in human urine samples for children, males, and females.

Location (sampling years)	Population	Age (years)	*N*	MP	EP	PP	BP	BP3	Reference
Belgium (2013)	**Children**	**1–11**	**48**	**18.6 (581.1)**	**1.1 (13.7)**	**1.1 (96.9)**	**0.5 (5.7)**	**1.6 (15.2)**	**This study**
Spain (2005-2006)	Boys	4	30	150.0 (—)	8.1 (—)	21.5 (—)	1.2 (—)	1.9 (—)	[37]
USA (2009-2010)	Children	6–11	415	26.5 (873.0)	<LOD (11.5)	2.7 (114.0)	<LOD (2.2)	14.6 (1570.0)	[38]
Denmark (2011)	Children	6–11	143	3.0 (62.0)	0.4 (3.7)	1.7 (33.0)	<LOD (1.4)	1.8 (40.0)	[39]
China (2012)	Children	9-10	70	— (—)	— (—)	— (—)	— (—)	0.6 (6.4)[‡]	[40]
Belgium (2013)	**Males**	**1–75**	**123**	**7.7 (223.4)**	**1.3 (41.4)**	**0.5 (20.2)**	**<LOD (4.9)**	**0.9 (34.5)**	**This study**
Denmark (2006)	Males	18–26	60	17.7 (2002.0)[‡]	2.0 (564.0)[‡]	3.6 (256.0)[‡]	0.2 (67.6)[‡]	— (—)	[41]
USA (2009-2010)	Males	≥6	1399	25.3 (727.0)	<LOD (36.4)	2.8 (134.0)	<LOD (2.7)	15.3 (610.0)	[38]
Belgium (2013)	**Females**	**1–85**	**138**	**32.4 (630.6)**	**1.9 (83.1)**	**3.3 (116.5)**	**0.5 (11.1)**	**1.7 (32.7)**	**This study**
France (2002–2006)	Pregnant women	—	191	104.3 (2689.7)	1.5 (38.2)	10.4 (267.7)	2.2 (63.6)	1.7 (143.0)	[42]
Spain (2004–2008)	Pregnant women	—	120	191.0 (—)	8.8 (—)	29.8 (—)	2.4 (—)	3.4 (—)	[37]
Japan (2007–2010)	Pregnant women	32.6[¥]	111	75.8 (1361.0)[‡]	7.5 (593.0)[‡]	20.2 (2690.0)[‡]	0.6 (22.8)[‡]	— (—)	[43]
USA (2009-2010)	Females	≥6	1350	106.0 (1230.0)	2.0 (138.0)	20.2 (361.0)	0.3 (31.8)	32.0 (3200.0)	[38]
Puerto Rico (2010–2012)	Pregnant women	—	105	153.0 (1590.0)	— (—)	36.7 (493.0)	0.4 (36.4)	31.3 (2150.0)	[30]
China (2010–2012)	Pregnant women	≥18	567	— (—)	— (—)	— (—)	— (—)	0.1 (0.8)	[44]
Danemark (2011)	Mothers	31–52	145	14.0 (275.0)	0.9 (44.0)	<LOD (14.0)	<LOD (9.3)	3.7 (312.0)	[39]

—: no results/information.
[‡]maximum.
[¥]mean.

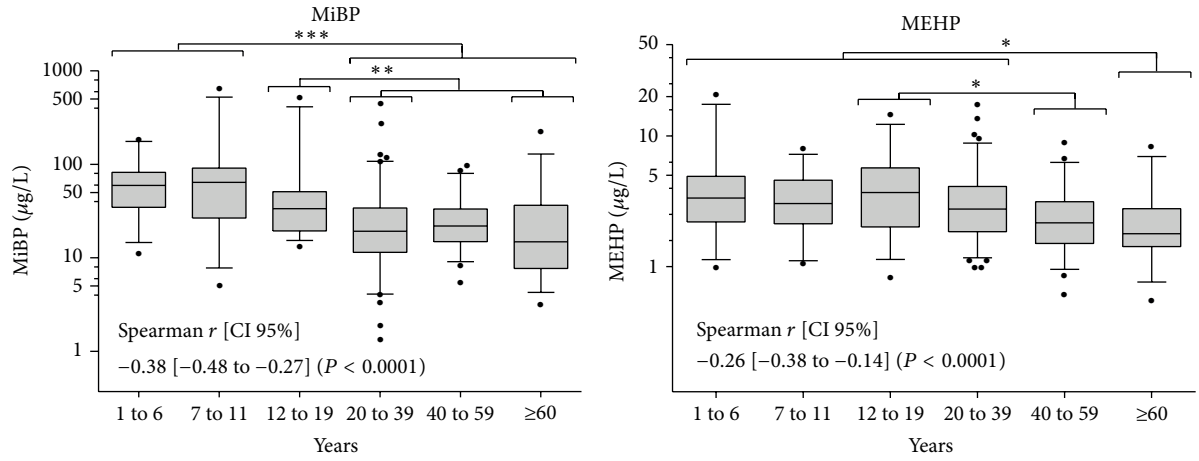

Figure 1: Urinary concentrations of MiBP and MEHP [μg/L] according to the age groups. The lower and upper boundaries of the boxes represent the 25th and 75th percentile, respectively. The line within the box is the median level and the whiskers are the 5th and 95th percentiles. Spearman's rank correlations [95% confidence interval] between metabolite concentrations and age are mentioned. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

and lower urinary concentrations than in some Danish and American children [38, 39]. On the other hand, the Belgian men seemed to be less exposed to all parabens than the Danish or American male populations [38, 41]. Focusing on women results, more data used to be available on urinary paraben levels and more specifically for pregnant women. In the present study, the levels measured in the women urine were overall quite lower than those reported in French, Spanish, Japanese, American, or Puerto Rican women but slightly higher than those described in some Danish mothers [30, 37–39, 42, 43]. It is of note that the lower BP levels and detection rate were also observed in all biomonitoring surveys, and even if the use of specific paraben according to the application could be variable between countries, the profiles were consistent in all studies with MP sharing for 75 to 90%, followed by PP and EP.

3.2. BP3. BP3 was detected in 82.8% of analyzed samples with levels ranging from <LOD to 662.8 μg/L and a GM level of 1.3 μg/L (Table 2). Similar to parabens, since BP3 used to be frequently incorporated in personal care products, its urinary levels were demonstrated to be correlated with the use of cosmetics [30]. Nevertheless, no significant difference was observed between males and females in the present study ($P = 0.086$) unlike in the NHANES study [36]. BP3 levels measured were significantly higher in adolescents (12–19 years) compared to adults (Table 3). This higher exposure for adolescents could not be reasonably explained. Besides the slight but significant correlation between BP3 and parabens ($r = 0.27$–0.37 $P < 0.001$) already observed in a previous study [30], BP3 seemed to be weakly correlated with some phthalate metabolites, mainly MnBP, MiBP, 5-OH-MEHP, and 5-oxo-MEHP ($r = 0.33$–0.37 $P < 0.001$). Although personal care products are known to be a source of exposure for both BP3 and parabens, other BP3 exposure routes have been suggested such as sunscreens or plastic surface coatings for food packaging [10, 31, 36]. We suspected this plastic food packaging to be a common route of exposure for BP3 and phthalates, therefore explaining the correlation found between both chemicals classes. On the other hand, the phthalates and BP3 are used in a wide range of other applications in the everyday life, and therefore a weak correlation was not unexpected [10, 61].

Compared to other surveys (Table 5), BP3 levels measured in the present study were fairly similar to those observed in child or adult population from different countries [37, 42] except in USA or in Puerto Rico [30, 36, 39] where levels found were up to 10- to 20-fold higher. This higher exposure would most likely be the reflection of the higher use of BP3 in North America where, for instance, 59% of the sunscreens were reported to contain this chemical [77]. Conversely, the urinary BP3 levels observed in China were much lower than those measured in Belgium [40, 44].

3.3. Phthalate Metabolites. The phthalate metabolites were positively detected in nearly all urine samples analyzed (Table 2). The GM ranged from 2.7 to 8.6 μg/L for MBzP, MEHP, 5-oxo-MEHP, and 5-OH-MEHP while higher levels were observed for MEP, MnBP, and MiBP with GM ranging between 26.2 and 37.6 μg/L. We did not observe any significant difference in urinary phthalate metabolite concentrations between males and females except for the sum of the metabolites of the diethylhexylphthalate (MEHP, 5-oxo- MEHP, and 5-OH-MEHP) which was statistically higher in men ($P = 0.0166$). The distribution of the metabolite levels is presented according to the age classification for MiBP and MEHP as an example in Figure 1. This figure details the significant differences which exist between the different age groups for both metabolites, while the global significant observations are shown in Table 3. As it was previously reported [47, 49, 78], the levels observed in children were quite higher than in adults. This reinforces the assumption raised by Silva et al. [78] about higher phthalate exposure for children relating to more time spent indoors and therefore the higher

Table 6: Phthalate metabolite concentrations—median (95th percentile) in μg/L—reported in human urine samples for children and adults.

Location (sampling years)	Population	Age (years)	N	MEP	MnBP	MiBP	MBzP	MEHP	5-OH-MEHP	5-oxo-MEHP	Reference
Belgium (2013)	**Children**	**1–11**	**48**	**35.6 (139.6)**	**55.7 (132.7)**	**61.8 (175.8)**	**9.7 (52.8)**	**3.1 (7.2)**	**18.7 (61.7)**	**12.3 (47.0)**	**This study**
Taiwan (2001-2002)[a]	Children	2–6	89	— (—)	87.9 (16455.0)[‡]	21.9 (252.7)[‡]	3.8 (69.4)[‡]	8.1 (94.7)[‡]	39.6 (1014.0)[‡]	31.0 (761.0)[‡]	[45]
Germany (2003–2006)	Children	3–14	599	— (—)	93.4 (310.0)	88.1 (308.0)	18.1 (76.2)	6.7 (25.1)	46.0 (164.0)	36.3 (123.0)	[46]
Spain (2005-2006)	Children boys	4	30	324.0 (—)	30.2 (—)	41.9 (—)	33.0 (—)	6.2 (—)	57.4 (—)	44.6 (—)	[37]
Canada (2007–2009)	Children	6–11	1037	23.6 (210.7)	32.6 (168.2)	— (—)	21.4 (131.1)	6.4 (17.8)	31.6 (179.5)	20.3 (106.7)	[47]
USA (2009-2010)	Children	6–11	415	33.0 (288.0)	23.3 (124.0)	10.9 (55.4)	12.6 (87.8)	1.7 (8.9)	17.0 (75.1)	11.1 (48.4)	[38]
Denmark (2011)	Children	6–11	143	20.0 (68.0)	32.0 (99.0)	54.0 (193.0)	7.0 (31.0)	2.0 (10.0)	23.0 (89.0)	12.0 (40.0)	[39]
Korea (2011)	Children	0–6	392	— (—)	— (—)	— (—)	— (—)	14.9 (58.1)	80.3 (253.2)	83.3 (265.5)	[48]
Belgium (2011-2012)	Children	6–11	125	23.0 (169.0)	40.0 (122.0)	54.0 (362.0)	8.6 (27.0)	2.2 (8.7)	17.0 (31.0)	13.0 (22.0)	[49]
Belgium (2013)	**Males and females**	**12–85**	**213**	**34.3 (396.3)**	**30.2 (142.2)**	**20.1 (89.3)**	**4.6 (26.5)**	**2.5 (8.7)**	**7.4 (30.6)**	**4.9 (19.1)**	**This study**
Sweden (2001)	Mothers	23–39	38	35.0 (761.0)[‡]	46.0 (198.0)[‡]	16.0 (130.0)[‡]	13.0 (38.0)[‡]	9.0 (57.0)[‡]	15.0 (126.0)[‡]	11.0 (83.0)[‡]	[50]
Taiwan (2001-2002)	Pregnant women	31–39	100	— (—)	52.4 (928.0)[‡]	10.3 (269.0)[‡]	1.2 (55.0)[‡]	10.5 (218.0)[‡]	21.7 (617.0)[‡]	20.8 (645.0)[‡]	[45]
The Netherlands (2002–2006)	Pregnant women	18–41	99	117.0 (1150.0)	42.8 (197.0)	42.1 (249.0)	7.5 (95.8)	6.9 (82.8)	14.0 (86.2)	14.5 (104.0)	[51]
Peru (2004)	Pregnant women	14–46	79	32.2[¶] (—)	9.3[¶] (—)	1.2[¶] (—)	1.1[¶] (—)	1.6[¶] (—)	4.1[¶] (—)	3.1[¶] (—)	[52]
Spain (2004–2008)	Pregnant women	17–43	120	755.0 (—)	27.5 (—)	29.9 (—)	10.5 (—)	4.4 (—)	17.3 (—)	15.7 (—)	[37]
Germany (2005)[b]	Males and females	14–60	399	— (—)	49.6 (171.5)	44.9 (182.6)	7.2 (45.6)	4.9 (21.7)	19.2 (21.7)	14.7 (56.0)	[53]
Japan (2005–2008)	Pregnant women	31.9[¥]	149	6.0 (1067.0)[‡]	48.1 (504.0)[‡]	— (—)	3.5 (992.0)[‡]	4.4 (70.3)[‡]	8.6 (89.7)[‡]	9.2 (132.0)[‡]	[54]
Israel (2011)	Males and females	20–74	248	— (—)	27.9 (90.8)[◊]	37.6 (89.0)[◊]	4.3 (20.5)[◊]	11.2 (49.3)[◊]	30.4 (91.1)[◊]	17.1 (55.5)[◊]	[55]
Mexico 2007	Females	32–79	108	83.2[¶] (—)	72.4[¶] (—)	8.4[¶] (—)	4.4[¶] (—)	5.2[¶] (—)	45.8[¶] (—)	31.8[¶] (—)	[56]
France 2007	Pregnant women	—	279	43.5 (600.7)	35.7 (201.1)	53.7 (274.1)	10.1 (88.7)	16.7 (266.6)	41.9 (605.1)	28.5 (427.9)	[57]
Denmark (2007–2009)	Males	19.5[¥]	881	78.0 (1936.0)	28.0 (91.0)	58.0 (173.0)	34.0 (164.0)	4.0 (18.0)	23.0 (79.0)	14.0 (55.0)	[58]
Canada (2007–2009)	Males and females	6–49	3236	49.1 (824.2)	23.8 (120.9)	— (—)	12.3 (81.9)	3.5 (24.9)	23.4 (180.3)	14.0 (113.8)	[47]
USA (2009-2010)	Males and females	≥6	2749	54.9 (988.0)	15.9 (75.9)	8.3 (41.3)	6.7 (48.3)	1.5 (14.1)	12.9 (103.0)	8.0 (55.7)	[38]
China (2010)	Males and females	10–40	183	21.5 (1330.0)[‡]	61.2 (798.0)[‡]	56.7 (791.0)[‡]	0.6 (43.0)[‡]	2.1 (207.0)[‡]	11.3 (1120.0)[‡]	7.0 (564.0)[‡]	[59]
Korea (2011)[c]	Males, females, and mothers	20–39	562	— (—)	— (—)	— (—)	— (—)	9.5 (94.0)	27.6 (98.2)	21.1 (82.3)	[48]
Denmark (2011)	Mothers	31–52	145	29.0 (359.0)	20.0 (70.0)	36.0 (139.0)	4.0 (22.0)	1.7 (6.9)	12.0 (50.0)	6.1 (21.0)	[39]
Belgium (2011-2012)	Mothers	≤45	125	34.0 (240.0)	31.0 (119.0)	33.0 (175.0)	6.4 (23.0)	2.3 (9.1)	11.0 (51.0)	7.6 (13.0)	[49]
Italy (—)[d]	Males and females	19–58	157	59.0 (748.6)	24.2 (143.0)	— (—)	16.7 (102.9)	3.1 (13.4)	12.1 (49.4)	— (—)	[60]

N: number of participants.
[‡]maximum.
[¥]arithmetic mean.
[¶]geometric mean.
—: no results/information.
[◊]Percentile 90th.
[a]Arithmetic mean of 2-3 and 5-6 years groups medians.
[b]Urine collected over eight consecutive days for each participant except one for seven days.
[c]12 h urine. Arithmetic mean of male, female, and mother medians.
[d]Arithmetic mean of female and male medians.

exposure to the phthalates potentially found in the household environment such as in carpets, vinyl flooring, pigments, or paints [79, 80]. Furthermore, children are known to have higher respiratory rates leading to higher exposure through indoor air and house dust [81, 82]. Their relatively higher food intake/body-weight ratio could also result in higher exposure than adults [78].

Some moderate to very high correlations were observed among the phthalate metabolites (Table 4). As expected, the three metabolites of diethylhexylphthalate (DEHP) were highly correlated ($r = 0.69$–0.96 $P < 0.001$). A stronger association between both oxidized metabolites of DEHP was observed compared to the correlation between oxidized metabolites and MEHP. This is consistent with some previous studies [83, 84], and one of the reasons for this lower correlation rate might be explained by the differences in half-time elimination between oxidized DEHP metabolites and MEHP [61]. MiBP, MnBP, and MBzP were moderately to highly correlated ($r = 0.60$–0.68 $P < 0.001$) but also with 5-oxo-MEHP and 5-OH-MEHP ($r = 0.54$–0.62 $P < 0.001$). MEP was moderately associated with MnBP and MBzP ($r = 0.51$ and $0.44 < 0.001$, resp.) but weakly with other phthalate metabolites ($r = 0.18$–0.32 $P < 0.001$). These results suggest that Belgians seemed to be exposed to some mixtures of phthalates through similar routes. Göen et al. [83] and Frederiksen et al. [85] also reported roughly comparable correlations between phthalate metabolites, but some correlation rates could slightly differ illustrating the variability of the exposure pattern of phthalates through the European countries.

During the past decade, numerous biomonitoring studies focused on the phthalate metabolites in the general population or in some specific subpopulations. A nonexhaustive comparison between different national large-scale studies in children and adults is presented in Table 6. The levels of the phthalate metabolites measured in the present study were fairly similar to those observed in the Belgian children and mothers recruited during the recent DEMOCOPHES study [49].

Focusing on the child population, the sum of the phthalate metabolites in the Belgian urine samples was comparable to those reported from Denmark [39], Taiwan [45], and Canada [47] but higher than in the CDC study [38] and lower in German [46] and Korean children [48]. Except in Spain where the highest phthalate metabolite levels were measured [37] either for children or adults, the levels measured in our adult participants seemed to be close to those reported in most of the other adult populations from Europe, Asia, or North America [38, 44, 47, 50, 54, 59, 60]. Nevertheless, quite higher urinary levels were reported in some studies such as in France, The Netherlands, Germany, or Mexico [51, 53, 56, 57] while very low urinary concentrations were measured in pregnant Peruvian women [52]. The urine of the present Belgian children and adults seemed to show a different phthalate metabolite profile, characterized by higher proportions of MnBP and MiBP compared to MEP for children and a higher MEP excretion rate for adults. This different profile could be related to a different exposure pattern for children and adults, with a higher exposure to diethyl phthalate due to higher use of personal care products by adults [86] compared to children. For the latter, the potential contamination of the interior environment could be considered as an important pathway of exposure [78]. These exposure patterns observed could be country dependent and probably related to different food or lifestyle habits and specific commercial use of phthalates. For example, the Chinese adults [59] showed higher MiBP and MnBP levels than MEP while the French or Mexican women [56, 57] presented a greater level of the metabolites of DEHP compared to other phthalate biomarkers.

4. Conclusion

This study reported for the first time, to the best of our knowledge, the simultaneous measurement of 7 phthalate metabolites, 4 parabens, and the BP3 in 261 participants from the Belgian general population aged from 1 to 85 years. Although this work presents several limitations in terms of representativeness such as a low sample number, limited sampling localization, and a small socioeconomic diversity, our results were close to the Belgian DEMOCOPHES references values [49]. As reported in other biomonitoring studies, we observed widely spread population exposure to these endocrine disruptor chemicals. The urinary paraben levels observed in the present study were statistically higher in women. Because the skin effects of alkyl parabens at environmental doses are still unknown, their potential interaction with CMM cells should be investigated considering that exposure for the women seemed to be higher due to the use of personal care products. EP, BP3, and phthalate metabolites (excepted MEP) showed significant different urinary levels according to the age groups. Higher exposure in younger age groups is a matter of concern since the disruption of hormonal balance during the development stage might have long-term consequences on their health. The results obtained in this study showed some important differences in terms of exposure levels and pattern among different countries but also among participants in the same population. The sum of the twelve targeted compounds ranged between 14.8 and 8575.2 μg/L showing that the cumulative exposure might be 600 times higher from one individual to another. This is also a matter of concern since additive endocrine disrupting effects are to be expected [87].

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Female Gender and Acne Disease Are Jointly and Independently Associated with the Risk of Major Depression and Suicide: A National Population-Based Study

Yi-Chien Yang,[1,2] Hung-Pin Tu,[3] Chien-Hui Hong,[4] Wei-Chao Chang,[5,6] Hung-Chun Fu,[2,7] Ji-Chen Ho,[1] Wei-Pin Chang,[8] Hung-Yi Chuang,[9,10] and Chih-Hung Lee[1]

[1] *Department of Dermatology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 83301, Taiwan*
[2] *Graduate Institute of Clinical Medical Sciences, Chang Gung University, College of Medicine, Taoyuan 33302, Taiwan*
[3] *Department of Public Health and Environmental Medicine, School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung 80756, Taiwan*
[4] *Department of Dermatology, Kaohsiung Veterans General Hospital and National Yang Ming University Hospital, Kaohsiung 81362, Taiwan*
[5] *Master Program for Clinical Pharmacogenomics and Pharmacoproteomics, School of Pharmacy, Taipei Medical University, Taipei 10478, Taiwan*
[6] *Department of Clinical Pharmacy, School of Pharmacy, Taipei Medical University, Taipei 10478, Taiwan*
[7] *Department of Obstetrics and Gynecology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan*
[8] *Department of Healthcare Management, Yuanpei University, Hsinchu 30015, Taiwan*
[9] *Department of Environmental and Occupational Medicine, Kaohsiung Medical University Hospital, Kaohsiung 80756, Taiwan*
[10] *Department of Public Health, Kaohsiung Medical University, Kaohsiung 80756, Taiwan*

Correspondence should be addressed to Hung-Yi Chuang; ericch@kmu.edu.tw and Chih-Hung Lee; dermlee@gmail.com

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Acne is a common disease in adolescence with female preponderance. It could cause poor self-esteem and social phobia. Previous studies based on questionnaires from several thousands of adolescents showed that acne is associated with major depression and suicide. However, the gender- and age-specific risk of depression and suicide in patients with acne remain largely unknown. Using a database from the National Health Insurance, which included 98% of the population of Taiwan in 2006, we identified patients of acne, major depression, and suicide based on ICD-9-CM codes. Totally 47111 patients with acne were identified (16568 males and 30543 females) from 1 million subjects. The youths of 7–12 years had the highest prevalence of acne (14.39%). Major depression was more common in those with acne (0.77%) than controls (0.56% , $P < 0.0001$) regardless of gender. Multiple logistic regression showed an increased risk of major depression in women without acne (OR = 1.85, 95% CI 1.75–1.96). The risk is additive in women with acne (OR = 2.78, 95% CI 2.43–3.17). Similar additive risk of suicide was noticed in women with acne. In conclusion, acne and gender, independently and jointly, are associated with major depression and suicide. Special medical support should be warranted in females with acne for the risk of major depression and suicide.

1. Introduction

Acne is a common skin disease in adolescence and can persist, in some cases, into adulthood [1, 2]. The facial appearance is important in social interaction, self-image, and self-esteem. Although acne is not a life-threatening disease, it could be distressing and cause adverse psychosocial consequences such as depression, poor self-esteem, and social phobia in the patients.

Major depression is recognized as a persistent low mood accompanied by low self-esteem, feelings of worthlessness, and a loss of general interest. Suicide attempt is an indicator

of emotional distress and tends to occur in patients with major depression. Major depression is a well-known major risk factor of suicide [3]. In Taiwan, suicide is a significant social problem as it is the third leading cause of death among the adolescent to the middle aged people [4].

As compared to men, in general, women have a higher chance of developing major depression, anxiety, and neurotic disorder and might have overall impaired quality of life [5]. Limited to the patients with acne, there is an increase of depressive symptoms in adolescents with acne [6–8]. In addition, several recent studies based on questionnaire survey have confirmed the association of acne with suicide attempts [7, 9] and body dysmorphic disorder [10]. In fact, an effective treatment of acne was accompanied by an improvement in self-esteem and mood [11].

Acne tends to affect females with a male to female ratio about 1/1.1–1.25 in Asians [12, 13]. We previously reported the epidemiological characteristics of acne and its associated disease burden in schoolchildren in Taiwan. Consistent with previous studies, more females are affected than males in our studies (M : F = 1/1.5–1.9) [14, 15]. In women, a high proportion of these acne cases are late onset [1, 2]. Although previous studies have found that acne is associated with the development of major depression and suicide, its impacts on the major depression and suicide may vary in people with different age, gender, and ethnic and cultural background. For example, most of the previous studies were limited to the adolescent populations. Large-scale studies regarding the association of acne and major depression and suicide from the childhood to adulthood are scarce. Moreover, although major depression and suicide tend to affect women and patients with acne, women are in fact more likely than men to develop acne. A question remains how acne by itself would be associated with the development of major depression and suicide. This makes it relevant to study the independent association between acne and major depression/suicide, as adjusted by genders and ages in a large population.

In this study, we aimed to determinate the age- and gender-specific prevalence of acne in Taiwan. In addition, we also evaluated the associations between acne and depressions and suicides. We also estimated the impact of female gender and acne disease on the risk of major depression and suicide.

2. Materials and Methods

2.1. Patient Ascertainment. This is a population-based study using the claim data collected by the National Health Insurance (NHI). Taiwan NHI is a program of health-care system. In 2006, more than 98% of Taiwan's population (about 23 million) was enrolled in this program. Therefore, our study population consists of almost the whole population of Taiwan in 2006. Based on the NHI database of year 2006, we collected and analyzed data related to acne, major depression, and suicide attempt as defined by the International Classifications of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes. Ascertainment of acne involves the selection of ICD-9-CM codes for acne including 706.1 with other acne, 706.2 with sebaceous cyst, 706.3 with seborrhea, 706.8 with other specified diseases of sebaceous glands, and 706.9 with unspecified disease of sebaceous glands. Further, we chose ICD-9-CM codes for major depression, including 296.2 with major depressive disorder single episode and 296.3 with major depressive disorder recurrent episode. ICD-9-CM codes for suicide attempt include the following: E950 with suicide and self-inflicted poisoning by solid or liquid substances, E951 with suicide and self-inflicted poisoning by gases in domestic use, E952 with suicide and self-inflicted poisoning by other gases and vapors, E953 with suicide and self-inflicted injury by hanging strangulation and suffocation, E954 with suicide and self-inflicted injury by submersion (drowning), E955 with suicide and self-inflicted injury by firearms air guns and explosives, E956 with suicide and self-inflicted injury by cutting and piercing instrument, E957 with suicide and self-inflicted injuries by jumping from high place, E958 with suicide and self-inflicted injury by other and unspecified means, and E959 with late effects of self-inflicted injury.

2.2. Statistics. The prevalence of acne and depression was stratified by sex and age group. The 95% CI of the prevalence was estimated by the Poisson distribution model. In the univariate analysis, the association between acne and the prevalence of major depression and suicide was tested using Fisher's exact test for categorical variables. The relationship between acne and prevalence of the major depression was assessed by logistic regression. Results are presented as odds ratios (OR) and 95% confidence intervals (95% CI). Interactions between acne and gender were tested under multiple logistic regression models using an added interaction term (acne × gender) and main covariates, age categories. A P value of <0.05 was considered significant. All statistical analyses were performed by using the software packages SAS, version 9.3 (SAS Institute Inc., Cary, North Carolina, USA).

3. Results

3.1. Age- and Gender-Specific Prevalence of Acne in Taiwan. To investigate the general prevalence rate of acne in Taiwan, we used the Taiwan National Health Insurance (NHI) database with a random sampling of one million patients visiting health care providers in 2006 to estimate the overall prevalence of acne (Table 1). Among these one million subjects, totally 47111 patients with acne (ICD-9-CM 706.x except 706.0) from the one million subjects at risk were identified (including 16568 male patients and 30543 female patients). Our result showed that overall prevalence of acne in Taiwan in 2006 was 4.71% (95% CI, 4.67%–4.75%). People aged 7–12 years (elementary school age) had the highest risk of developing acne, with a prevalence rate at 14.39% (95% CI, 14.15%–14.63%). The prevalence decreased with age thereafter, but there were a significant number of patients experiencing acne after 19 years old, especially in females aged 19–42 years. Acne was relatively uncommon in people older than 42 years of age. To determine whether women were prone to acne, we further stratified the prevalence of acne by gender. The prevalence was higher in women, at a rate of 6.06% (95% CI, 5.99%–6.12%), than in men, at a rate of 3.34 (95% CI, 3.29%–3.39%). Male to female ratio of prevalence is around 1 : 1.81. Girls aged 7–12 years had the highest prevalence rate of acne (17.78%,

Table 1: Prevalence of acne in Taiwan, 2006.

	ICD-9-CM 706.x	At risk population	Prevalence (95% CI)
	n	*n*	
Men			
Overall	16568	495838	3.34% (3.29%–3.39%)
Age category, years			
0–6	4234	110114	3.85% (3.73%–3.96%)
7–12	4291	39800	10.78% (10.48%–11.09%)
13–18	2732	46295	5.90% (5.69%–6.12%)
19–24	1482	48014	3.09% (2.94%–3.25%)
25–30	956	51107	1.87% (1.76%–1.99%)
31–42	1355	96541	1.40% (1.33%–1.48%)
>42	1518	103967	1.46% (1.39%–1.54%)
Women			
Overall	30543	504162	6.06% (5.99%–6.12%)
Age category, years			
0–6	4711	100662	4.68% (4.55%–4.81%)
7–12	7549	42469	17.78% (17.42%–18.14%)
13–18	7098	50167	14.15% (13.85%–14.46%)
19–24	4184	51782	8.08% (7.85%–8.32%)
25–30	2957	54410	5.44% (5.25%–5.63%)
31–42	2859	98842	2.89% (2.79%–3.00%)
>42	1185	105830	1.12% (1.06%–1.19%)
Combined group			
Overall	47111	1000000	4.71% (4.67%–4.75%)
Age category, years			
0–6	8945	210776	4.24% (4.16%–4.33%)
7–12	11840	82269	14.39% (14.15%–14.63%)
13–18	9830	96462	10.19% (10.00%–10.38%)
19–24	5666	99796	5.68% (5.54%–5.82%)
25–30	3913	105517	3.71% (3.60%–3.82%)
31–42	4214	195383	2.16% (2.09%–2.22%)
>42	2703	209797	1.29% (1.24%–1.34%)

95% CI 17.42%–18.14%). In addition, in each age category, women were at higher risk than men of developing acne. In contrast to the decreasing trend of prevalence in men after the age of 13, women at high school age (age 13–18) remained at a higher risk of developing acne, reaching a prevalence at 14.15% (95% CI, 13.85%–14.46%).

3.2. Patients with Acne Tend to Develop Major Depression and Commit Suicide in Both Genders. To further define the impact of gender in the development of major depression and suicide attempt in patients with acne, we first performed the association analysis of acne and major depression/suicide as stratified by the gender. The overall prevalence of major depression and suicide attempts in patients with acne was 0.77% and 0.01%, respectively (Table 2). The result demonstrated that major depression (0.77%) was more common in those with acne than controls (0.56%, $P < 0.0001$). We also observed an increased risk of suicide among patients with acne although it did not reach a statistical significance. In general population, the prevalence of major depressions is more common in women than those in men at about 1.5-fold [5]. Concurrently, the prevalence of major depression and that of suicide are both higher in women with acne than those in men with acne. Taken together, our data confirmed that patients with acne tend to develop major depression in both genders and suggested that genders might affect the risk of developing major depression in patients with acne.

3.3. Acne and Gender, Independently and Jointly, Are Associated with the Risk of Major Depression and Suicide. We have shown that patients with acne and females are both associated with an increased risk of major depression and suicide (Table 2) as adjusted by age with a multiple logistic regression model. To further determine whether gender and acne both contributed to the risk of major depression, we performed further multiple logistic regression models using an added

Table 2: Patients with acne tend to develop major depression and commit suicide in both genders.

	ICD-9-CM 706.x *n*	Non-ICD-9-CM 706.x *n*	*P*
Men			
Major depression (296.2 + 296.3)			
+	101 (0.61)	1851 (0.39)	<0.0001
−	16467 (99.39)	477419 (99.61)	
Suicide (E950–E959)*			
+	1 (0.01)	23 (0.00)	0.5577
−	16567 (99.99)	479247 (100.00)	
Women			
Major depression (296.2 + 296.3)			
+	261 (0.85)	3453 (0.73)	0.0142
−	30282 (99.15)	470166 (99.27)	
Suicide (E950–E959)*			
+	6 (0.02)	45 (0.01)	0.1281
−	30537 (99.98)	473574 (99.99)	
Combined group			
Major depression (296.2 + 296.3)			
+	362 (0.77)	5304 (0.56)	<0.0001
−	46749 (99.23)	947585 (99.44)	
Suicide (E950–E959)*			
+	7 (0.01)	68 (0.01)	0.0900
−	47104 (99.99)	952821 (99.99)	

*Fisher's exact test.

Table 3: Acne and gender, independently and jointly, associated with the risk of major depression.

	Major depression	Nonmajor depression	Adjusted OR (95% CI)*	β, *P* for interaction
Acne (no) and men	1851 (32.67)	477419 (48.01)	1.00	
Acne (no) and women	3453 (60.94)	470166 (47.28)	1.85 (1.75–1.96)	
Acne (yes) and men	101 (1.78)	16467 (1.66)	2.12 (1.73–2.60)	
Acne (yes) and women	261 (4.61)	30282 (3.05)	2.78 (2.43–3.17)	−0.35, 0.0043

*Adjusted age categories.

interaction term (acne × gender) and main covariates as age categories (Table 3). Our results showed that both acne and women are associated with the increased risk of major depressions. Without acne, women have an odds ratio of 1.85 (95% CI 1.75–1.96) to develop major depression as compared to men. In men, men with acne have a 2.12-fold (95% CI 1.73–2.60) increased risk of developing major depression than men without. The effects of women and acne in development of major depressions are additive since women with acne have a 2.78-fold (95% CI 2.43–3.17) increased risk of developing major depression (1.85 + 2.12-1~2.78).

Similarly, our results showed that both acne and women are associated with the risk of suicide (Table 4). Without acne, women have an odds ratio of 1.96 (95% CI 1.18–3.23) to commit suicide as compared to men. In contrast, men with acne do not have an increased risk of suicide than men without (OR = 1.01, 95% CI 0.15–8.24). In patients with acne, however, women with acne showed an overall increase of risk of suicide by 3.17-fold (95% CI 1.27–7.94). Special attention should be paid to the female patients with acne in terms of their particular risk of committing suicide.

4. Discussions

This study analyzed data from a large random sample of the general population in Taiwan. Acne was commonly thought to be only a skin problem that usually affected the adolescence. This study indicated the 7–12-year-old children being affected at the higher rates than adolescents (13–18 years old) and many adults also experience acne. In addition, females were more vulnerable to acne than males in all age groups. Consistent with our previous community-based study conducted by dermatologist's direct inspection and diagnosis [15], the trend of female predominance in this age group was observed. However, the prevalence of acne in school children was higher (M/F = 12.3%/23.4%) in the

Table 4: Acne and gender, independently and jointly, associated with the risk of suicide.

	Suicide	Nonsuicide	Adjusted OR (95% CI)*	β, P for interaction
Acne (no) and men	23 (30.67)	479247 (47.93)	1.00	
Acne (no) and women	45 (60.0)	473574 (47.36)	1.96 (1.18–3.23)	
Acne (yes) and men	1 (1.33)	16567 (1.66)	1.10 (0.15–8.24)	
Acne (yes) and women	6 (8.00)	30537 (3.05)	3.17 (1.27–7.94)	0.39, 0.7280

*Adjusted age categories.

previous study [15] than that in the current study (M/F = 10.78%/17.78%). This might result from the fact that not all the patients with acne seek medical help. The current study only included patients who had sought treatment for their acne. Therefore it may have underestimated the total prevalence of acne in the general population. Interestingly, based on these two studies with different experimental setup, prevalence of acne in males is similar (12.3% versus 10.78%), but a subtle difference existed in females (23.4% versus 17.78%). Therefore, the dermatologists and pediatricians should provide earlier medical, educational intervention to these young patients and their parents to improve the awareness of the management of acne and prevent the sequelae such as permanent scarring and the comorbid psychosocial illness.

Clearance of acne was generally expected to occur spontaneously in early adulthood. However, acne remains a common skin disease after the second decade [1, 16, 17]. Our results also demonstrated that acne lesion can persist into middle age for both genders. Females were at higher rate of 2-fold greater than males. This is consistent with the findings of female preponderance from a previous study in adult acne based on the participant's own perception of acne [1]. The current study and studies from others all found that females with acne were positively correlated with the psychological damages [18–20]. Thus, assessing psychological distress among the women with adulthood acne would be warranted.

We found that major depression was more prevalent by 2-fold in patients with acne (0.77%) than general population in Taiwan (0.35%) [5]. Furthermore, the risk of major depression was significantly higher in people with acne than those without ($P < 0.0001$). Previous studies have reported that acne could lead to psychological problems and it affects more females than males [8, 13, 18, 21, 22]. Similar impacts of acne on psychological impairment in both genders have been also demonstrated [9]. Our results indicate that the female gender and acne are two independently contributory factors to developing major depression.

The significance of the association of acne and major depression was higher in males and there was 2.12-fold increased risk of developing major depression in males with acne than those without. This claim data did not include disease severity as an outcome. Since the disease severity of acne is associated with social impairment [9], a possible explanation for the higher prevalence of major depression in females with acne but a more significant association of acne and major depression in males might be related to the higher severity of acne in school students aged 4–18 year old [23] and postadolescent [16].

Regarding the association of suicide and acne, an increased risk of suicide attempts in young people presenting with acne has been reported [7, 9, 24]. Although the association of acne and suicide did not reach statistical significance in this study, we observed the trend of increased risk of suicide among patients with acne. The impact of acne on different genders remains controversial. A questionnaire-based study among adolescents indicated that the impact of acne on suicidal ideation was equivalent in both genders [9]. However, among our male patients, the impact of acne on suicide was very limited, which was consistent with a previous study [25]. In this study, female gender and acne were two independent risk factors of suicide. Nevertheless, some authors have proposed mental distress as a possible cause of acne. Further studies focusing on the causal relationship of acne and these psychiatric problems remained to be investigated.

Isotretinoin is an effective therapeutic option for severe and recalcitrant acne. Use of isotretinoin was reported to be associated with depression and suicide attempts in patients with acne [26–28] although the direct casual effect in remains obscure. In the contrary, some recent studies showed that isotretinoin has favorable effects on depression and suicide attempts subsequently after successful treatment of acne [29–32]. The effect of isotretinoin on the depression and suicide becomes controversial. We tried to use the current study design to answer whether isotretinoin affected the development of major depression and suicide. However, from the NHI database, only 18 from 47111 patients with acne took isotretinoin. This underestimation of isotretinoin use resulted from the fact that isotretinoin reimbursement was limited to the patients with refractory nodular cystic acne based on the policy of NHI and the majority of the acne patients treated with isotretinoin paid out of their own pockets. Thus, we could not address whether the isotretinoin affected depression or suicide due to the limitation of the NHI database in this study.

Our study highlighted that acne is an early onset and chronic skin disease which may influence mental health throughout lifetime, especially in females. Acne could not be considered simply as a superficial problem in physical appearances. The requirement of active screen for the comorbidities of psychological diseases, such as major depression and suicide, among the acne patients should be warranted.

Although the numbers of patients with acne identified are tens of thousands, there are some intrinsic limitations using the NHI database. Firstly, the doctors may not give an accurate ICD-9-CM codes upon visit. Patients with different ages, genders, and diseases may have different thresholds to

visit doctors. Nonetheless, in this study, we still observed consistent female to male ratios and prevalence in patients with acne as compared to our previous studies, suggesting that the analysis based on NHI database may reflect at large the de facto situation. Secondly, the NHI database and the insurance forms could not address whether the females seek medical help earlier or the females were affected by the acne earlier. However, our previous community-based study indicated that comedones were actually identified in girls at age 6, which was 1 year earlier than that in boys. Thirdly, we could not distinguish reactive or endogenous depressions using this database. Further hospital-based case control study focusing on the association of acne and reactive/endogenous depression is needed.

We concluded that female gender and acne disease are jointly and independently associated with the risk of major depression and suicide. The results may provide useful decision making information to the public health policy decision makers to estimate the need of appropriate health care for patients with acne in our community. Education and encouragement of the people with acne, women in particular, to seek an appropriate medical, mental, and social support not only improve the physical appearance and self-esteem but also decrease the associated social consequences and burdens.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Yi-Chien Yang and Hung-Pin Tu have equal contributions, and Hung-Yi Chuang and Chih-Hung Lee have equal contributions.

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Review Article

Vulvar Skin Disorders throughout Lifetime: About Some Representative Dermatoses

Jean Doyen,[1] Stéphanie Demoulin,[2] Katty Delbecque,[3] Frédéric Goffin,[1] Frédéric Kridelka,[1] and Philippe Delvenne[3]

[1] *Department of Gynecology and Obstetrics, Regional Hospital Citadelle, 4000 Liege, Belgium*
[2] *Laboratory of Experimental Pathology, GIGA-Cancer, University of Liege, 4000 Liege, Belgium*
[3] *Department of Pathology, University Hospital of Liege, 4000 Liege, Belgium*

Correspondence should be addressed to Philippe Delvenne; p.delvenne@ulg.ac.be

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The objective of this paper is to present general considerations which should be kept in mind by clinicians in charge of women with vulvar diseases. Four representative vulvar dermatoses are described. Lichen simplex chronicus is a pathological condition related to chemical and mechanical irritant agents. Detrimental effects of these irritants, in the presence of other dermatoses, have to be considered when therapeutic responses are unsatisfactory. Lichen sclerosus is the most common vulvar dermatosis in elderly. However, it should be kept in mind that it may be diagnosed at any age. Lichen planus, in spite of sharing a similar range of etiological factors with lichen sclerosus, is a very distinct entity. Finally, Paget's disease, although rare, is also described especially because of the challenge it represents both clinically and therapeutically.

1. Introduction

Vulva is, for many reasons, to be regarded as a particular anatomic area. Considering anatomy and patient's own point of view, this region is of course not easily self-observable and, as a part of genitalia, often quite unknown and mysterious, for cultural or emotional reasons. It is composed of several folds including clitoral hood, labia majora and minora, hymen, and anal margin. Microscopically, vulva is covered by different types of epithelia, depending on the area of interest, including, from its lateral to medial region, keratinized hair bearing skin, partially keratinized hairless skin, and, beyond Hart's line, mucous membrane of the vestibule. A large number and variety of adnexal structures are associated with vulvar skin in its different sections, such as pilosebaceous units, sebaceous and sweat glands, mucous secretory glands, muscle fibers, and deeper major or minor vestibular glands. Vicinity of underlying vascular structures can also modify vulvar aspects. Therefore, any component of blood and lymphatic vessels can be affected through malformations, tumors, or dystrophic changes.

From a pathological point of view, vulva, as a part of genitalia, can be affected by specific disorders such as multifocal HPV lesions of any degree or vulvar expression of a vaginal infection. Vulva can also exhibit specific dermatological diseases for which signs can be observed elsewhere on the body, such as in lichen sclerosus or psoriasis. However, vulva can also exhibit signs of a large variety of diseases, such as digestive, hematological, immunological, and endocrine disorders. This leads us to consider any vulvar disorder as a potential expression of a very large panel of diseases.

Clinically, if many vulvar lesions are reasonably characteristic, numerous clinical manifestations are not specific of one disorder and some diseases can express different morphological patterns. For these reasons, the revision should follow unexpected no-response to empirical treatment resulting from a clinical diagnosis. Frequently, pathological patterns cannot be automatically related to one single cause.

Biopsy is certainly an important diagnostic step in many circumstances. As biopsy is an invasive procedure, especially on the vulva, special care should be taken. Unless for very suspicious lesions that require prompt diagnosis, topical

treatments, especially corticosteroids, should be stopped 3 to 4 weeks before performing biopsy to allow natural histological expression of the disease. Local anesthesia is mandatory and biopsy should be performed using a 4 to 5 mm punch device to avoid crushing artifacts occurring with biopsy forceps.

As a consequence of these considerations, it seems quite difficult to present a classification of disorders affecting vulva which all medical specialties would agree with. Classification of the Internal Society for the Study of Vulvar Diseases appears credible as it is periodically revisited and as it is the result of consensus between gynecologists, dermatologists, and pathologists. At the present time, 2006 ISSVD Classification [1] is still relevant. But, as this classification is of minor help for diagnosis, ISSVD formulated in 2011 a complementary classification as an approach to clinical diagnosis [2].

Clinicians dealing with vulvar complaints should always keep in mind these preliminary considerations and, as a consequence, be convinced that treating vulvar disorders needs a complete anamnestic investigation, examination of the lower genital tract, skin, and sometimes oral mucosae, and dialog between colleagues. Furthermore, special attention should be paid to psychosexual status of patients suffering from vulvar disorders, as they are often present, either as a cause or a consequence of the disease. Effect of vulvar diseases on self-regard, affective, and sexual life is potentially important. Therefore, time should be given for exhaustive explanations about etiology, nature, and course of the disease and consequences, if any, on sexual life. Causal treatment should also be associated with protective measures avoiding contacts with mechanical and chemical irritants. A nonexhaustive list of common recommendations includes avoiding fabric softeners, pads, detergents, cosmetic products containing color additives and flavors, and synthetic underwear. This is sometimes sufficient to eradicate irritant and contact dermatitis and limits risk of poor response to true dermatoses. In addition, any coexisting disorder, such as diabetes mellitus or urine incontinence, should be under control.

Too often, women suffering from vulvar complains, especially itching, are still nowadays offered symptomatic treatment without diagnostic process or even without examination, such as over-the-counter delivery of any cream. This may, in some cases, lead, even in young people, to delayed diagnosis of life-threatening diseases, such as squamous cell carcinoma (SCC). For this reason, in the presence of vulvar complains, a diagnostic pathway including systematic physical examination is always mandatory.

This raises the question of vulvar screening. Even if vulva shares some diseases with cervix and vagina, especially through HPV dependent lesions, the place of screening is not comparable. Indeed, vulvar cancer is ten times less frequent than cervical one. Most vulvar lesions are symptomatic, and vulva is accessible to observation. As a consequence, vulvar cytology development has a minor place, if any. On the contrary, vulvoscopy has to be regarded as a valid tool, especially in complicated clinical presentations and in situations where there is a risk of intraepithelial neoplasia or microinvasion [3].

Of course, this paper does not pretend to overview all the aspects of vulvar diseases. Consequently, selecting some of the most representative vulvar dermatoses is subjective. The four selected vulvar diseases described below illustrate the variety of pathologies which may be encountered and for which awareness is necessary.

Lichen simplex chronicus, a nonspecific disorder, may be the expression of a so common contact dermatitis but may also reveal another underlying disease. Lichen sclerosus is the most current vulvar dermatosis in elderly and often easy to treat, requiring a sustained attention. Lichen planus, in spite of having some common points with the previous one, is clearly distinct and often more difficult to evaluate. Finally, vulvar Paget's disease remains a pathological condition difficult to handle because it is clinically nonspecific, rare, and easily missed and for which new treatments are necessary.

2. Lichen Simplex Chronicus

This disorder is a chronic eczematous condition characterized by intense pruritus. Rubbing and scratching produce poorly demarcated plaques of thickened lichenified skin. Scale may be subtle and results in slightly shining. Erosions and fissures can result from scratching and become infected.

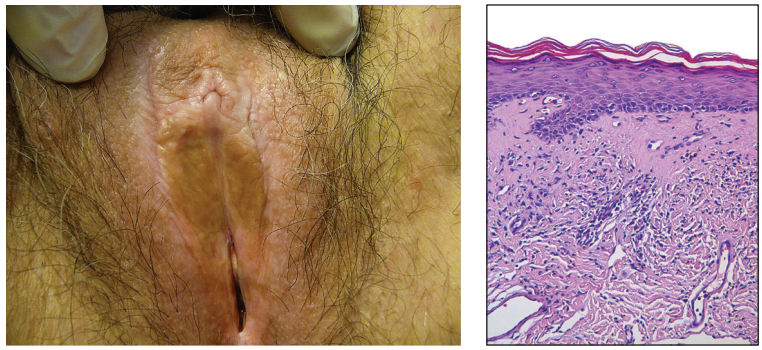
In children, atopic dermatis can result in lichen simplex chronicus, most often characterized by a widespread inflammatory eruption instead of vulvar lesions. Adults are more likely to present genital eczema.

Treatment requires a three-steps process. The first step consists in identifying and eliminating all irritant and allergen exposures. Secondly, treatment requires the breaking of the itch-scratch-itch cycle, especially during nighttime sedation, with oral antihistamine drug taken at bedtime and high potency topical corticosteroid, once a day at bedtime. Additionally, identifying and treating concomitant infection are needed.

Clinical control after one month is recommended. In some cases, another underlying pruritic condition might be revealed. If treatment fails or lichen simplex chronicus recurs, attention should be paid to psychological status of the patient. Psychological disturbances may as well be a cause or a consequence of persisting or recurring lesions. They should be treated specifically with appropriate support. As a result, surgical resection of a lichenified prominent labia majora should be an exceptional procedure. Pathologic analysis is of course necessary.

3. Lichen Sclerosus

Lichen sclerosus is one of the most common dermatoses affecting vulva in elderly. The disease is sometimes asymptomatic, but pruritus is frequent and represents by far the main complaint. However, depending on the way disease has developed, other complaints may be expressed. Pain may result from fissures and induce, for example, dyspareunia or constipation when perianal. Dyspareunia may also be due to introitus narrowing.



(a) (b)

Figure 1: (a) Irregular white to brown patch on the vulvar skin of a 74-year-old woman. (b) Representative example of a lichen sclerosus biopsy specimen showing a thinned epidermis with slight hyperkeratosis, loss of the rete ridges, and a band of homogenized collagen below the dermoepidermal junction.

Pathology is characterized by epithelial thinning down, sclerosis of the upper derma, and inflammatory infiltrate underneath (Figure 1(b)). Hyperkeratotic changes may also occur in some locations. Thin epithelium was previously thought to represent an atrophic state but is probably due to accelerated maturation. For that reason, this disease is no more called lichen sclerosus atrophicus. As a consequence, androgen-containing creams are no more up to date treatments, as they are not justified and as they induce virilization, locally (clitoral hypertrophy), or, sometimes, with general signs.

Clinically, vulvar lichen sclerosus induces mainly whitening of the skin, due to sclerosis (Figure 1(a)). Topography is variable from focal to large bilateral extension and from periclitoral to perianal area. Labia minora are often affected, either thickened or reduced. Clitoral hood may merge, bury clitoris, and result in pseudocyst formation, containing smegma. Sclerosis of the introitus may result in sexual impairment and dyspareunia. Inflammatory stages during which pruritus may be especially present can induce some degree of stromal congestion and skin redness, fissuring, and hyperkeratotic reaction. Thinning of the skin, combined with itching, results in petechia. Dystrophy and chronic irritation are probably a favorable condition for inducing nuclear atypia in the deepest layers of epithelium, resulting in high grade vulvar intraepithelial neoplasia (VIN) of a differentiated type. As a consequence, most vulvar SCC of elderly occur on dystrophic vulva and result from degenerating focal VIN. Vagina is never affected. On the other hand, extragenital manifestations can be observed.

Etiology of lichen sclerosus is unknown but related to an autoimmune mechanism. Other autoimmune diseases occur in 21% of patients: thyroiditis (12%), alopecia areata (9%), and vitiligo (6%) are the most frequent ones. It is more frequent in women than in men. Genital localization can occur at all time from prepubertal age to elderly and is more common at these ages than in middle ages [4].

In children, lichen sclerosus may be difficult to diagnose in some instances, or diagnosis is simply not expected. This should explain why mean interval between symptoms and diagnosis is reported to be around 1.7 years. At this period of life, vulvar folds are not completely developed, and vulvar folds changes nearly do not appear. Whitening may be present. Fissures can be nearly the only manifestation, especially around anus, inducing pain and constipation. These children may be suspected to be victim of sexual abuse, and this should be carefully taken in consideration. Furthermore, lichen sclerosus and abuse may coexist, the second triggering the first one. For that reason, any unexplained observation such as poor response to treatment should raise special attention [4]. Any parasitic origin such as oxyurosis should also be considered.

In young women, clinical presentation is not especially different than in elderly, but special attention should be paid to long-term risk of atrophic changes due to their effect on sexual life.

In elderly, the most important part of the treatment is the control of pruritus. But sexual complains are not rare, because long-term progression of the disease, added to natural atrophic changes of lower genital tract, alters vestibular anatomy and function. Periodic examination of the vulva under treatment remains important at this age, even in the absence of complain, because any persistent dystrophic area should be suspected to bear epithelial atypia and represent a VIN focus.

Occurrence of SCC is debated in its causative relation to lichen sclerosus. Although this cancer occurs in no more than 5% of lichen sclerosus. In contrast, histologic signs of lichen sclerosus are present in 33% of cases of SCC of the vulva [4]. Older age and epithelial hyperplasia are independent risk factors. The presumed pathway would imply successively lichen sclerosus, hyperplasia, and lower atypia representing differentiated type VIN, then leading to carcinoma. However, this sequence is not well understood and even controversial [5].

Considering hypertrophic forms of lichen sclerosus as a potential precancerous disease is not unanimously admitted. In a short series of patients with hyperplastic changes in lichen sclerosus, in whom followup was available for at least 5 years, no progression to cancer was noticed [6]. This

might be taken into consideration to avoid unuseful surgery. However, biopsy is necessary to assess the absence of atypia and observation is recommended in foci of hyperplastic epithelium in lichen sclerosus.

The presence of epithelial atypia in association with lichen sclerosus is also controversial. It is commonly admitted that VIN associated with lichen sclerosus is a differentiated type and that overexpression of p53 is present, contrary to what happens in undifferentiated type VIN, induced by HPV, and in which overexpression of p16 is encountered [5]. Once again, common link between differentiated type VIN and lichen sclerosus is debated [6], and at least one report previously showed that VIN associated with lichen sclerosus is most often of the undifferentiated type [7].

These data illustrate how it is difficult to obtain agreement between members from one or from different communities. This, in turn, affects dialog between clinicians and pathologists and sometimes makes surgical decisions difficult to discuss.

Treatment of lichen sclerosus is nearly always based on the use of potent corticosteroid cream. Application in the adequate area is to be teached, especially in elderly, because the area is not always easy to reach. Patients have to learn the way cream must be applied until complete skin absorption. Treatment effect has to be followed up on both complaints and objective vulvar appearance. This allows detecting wrong appliance of treatment or nonresponding suspect areas. Treatment must be adjusted, and applications reduced to minimal frequency and amount of cream needed. This reduces secondary effects risk of long-term use and leaves therapeutic margin if disease gets more active later. When disease is totally under control, stopping treatment for sometimes long periods should be debated. It is certainly indicated in younger patients and has to be discussed in older ones, who should always be followed up on a long-term basis, either treated or not, even if objective cancer occurrence risk is lower than 5%.

The place of topical calcineurin inhibitors in the treatment of lichen sclerosus and other conditions such as lichen planus is worth thinking about.

Topical calcineurin inhibitors are to be considered as a second-line treatment, when all reasonably possible adjustments of corticoid creams have been tested [8]. In some instances, corticoid creams are not or no more tolerated (delayed-type hypersensitivity reactions) or become less efficient, leading to increasing treatment doses (tachyphylaxis). They may also induce rebound erythroderma or lead to cutaneous atrophy. The main advantages of calcineurin inhibitors are to avoid tachyphylaxis and cutaneous atrophy. Nevertheless, they are associated with secondary effects. Topical calcineurin inhibitors commonly induce transient local burning or irritation. Facial flushing after alcohol ingestion and rosaceiform dermatitis may also less commonly occur [9].

Long-term safety profile of topical calcineurin inhibitors has been questioned, especially as they have been suspected to increase cancer risk. However, it seems reasonable to assess that this risk has been overestimated. It is, in fact, not higher than in the general population [10].

It has been demonstrated that potential systemic effects of topical calcineurin inhibitors are lower than that of corticosteroids, due to their weaker permeation potential. For that reason, lymphoproliferative changes observed in monkeys did not result in a higher risk in patients. Risk of immunosuppression was not documented by any increased risk of systemic or skin infections. Photocarcinogenicity was not described in patients using topical regimens [9]. Eventually, SCC occurring in the presence of topical calcineurin inhibitors was investigated by the US FDA. Twenty nine cases were reported worldwide and only 3 occurred in cases of lichen sclerosus [4].

Considering all these aspects, topical calcineurin inhibitors should be regarded as a second-line treatment. It has to be presented as an off-label option, if possible for short durations, owing to previous considerations and to their costs, clearly higher than that of corticosteroids [4].

Surgical treatment is limited to some indications: surgery of clitoral hood when clitoral phimosis is sexually detrimental or when a painful pseudocyst has developed; enlargement of introitus when superficial dyspareunia occurs; resection of dystrophic zones when biopsy demonstrates associated VIN or vulvectomy in the presence of carcinoma.

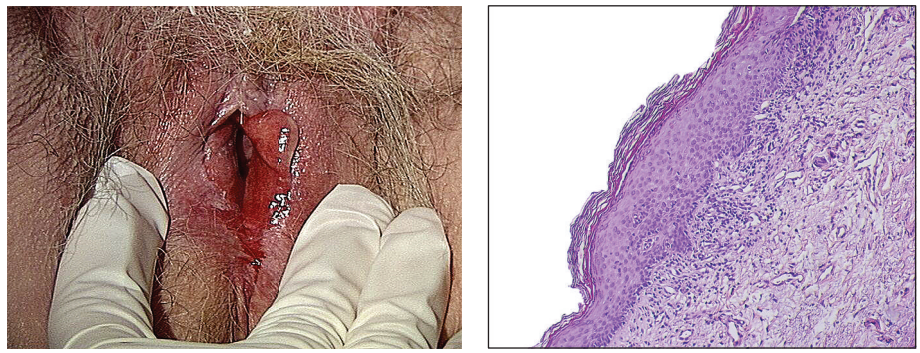
4. Lichen Planus

Lichen planus (Figure 2) is a more uncommon condition that may be asymptomatic, itchy, or more frequently painful. This is mainly due to frequent erosive forms, especially those affecting vestibulae. Chronic vaginal discharge and dyspareunia are frequently associated.

Pathology may show different patterns, in relation to different clinical appearances. Epidermis can be thickened or erosive. Basal cell layer shows some areas of vacuolar degeneration. A band-like mononuclear infiltrate is present in the upper dermis (Figure 2(b)).

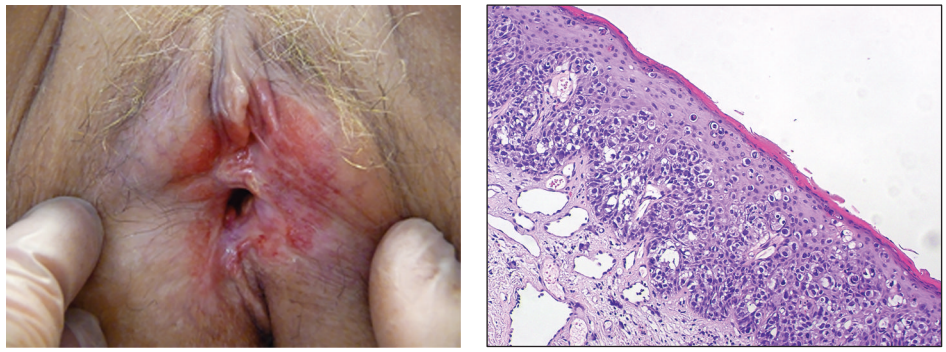
Clinically, vulvar lesions can be represented by different patterns, including violaceous shiny papules, reticular white networks, or erosive forms, especially on the vestibulae and vagina (Figure 2(a)). Actually, in contrast to lichen sclerosus, it can extend to vagina and turn, in this location, to extremely invalidating and difficult to treat erosive vaginitis. Extragenital and buccal locations are frequent. Erosive vulvovaginogingival lichen planus is known as the syndrome of Hewitt and Pelisse.

Consensus on the diagnostic criteria for erosive vulvar lichen planus was recently defined by members of Vulvar Diseases Societies [11]. Nine criteria were gathered of which three should be present to support the diagnostic: (i) well-demarcated erosions/erythematous areas at the vaginal introitus; (ii) presence of a hyperkeratotic border to lesions and/or Wickham striae in surrounding skin; (iii) symptoms of pain/burning; (iv) scarring/loss of normal architecture; (v) presence of vaginal inflammation; (vi) involvement of other mucosal surfaces; (vii) presence of a well-defined inflammatory band involving the dermoepidermal junction; (viii) presence of an inflammatory band consisting predominantly of lymphocytes; and (ix) signs of basal layer degeneration.



(a) (b)

Figure 2: (a) Wide vestibular erythematous erosive lichen planus in a 79-year-old woman. (b) Representative example of a lichen planus biopsy specimen showing an irregular hyperkeratosis and a lymphoid dermal infiltrate appearing as a distinct band close to the epidermis.



(a) (b)

Figure 3: (a) Wide red area extending mainly on the left side of the vulva in a 65-year-old woman with Paget's disease. (b) Representative example of Paget's disease biopsy specimen showing many glandular neoplastic cells with clear cytoplasm present singly or in small nests within the epidermis.

Etiology is, as for lichen sclerosus, suspected to be autoimmune. Moreover, in some cases, mixed forms of lichen planus and sclerosus are encountered.

This disease is rare in younger and older patients. Most of affected people are middle aged around 30 to 60 years.

Treatment is targeted against complains. Pruritic lesions respond to local applications of highly potent corticoid creams, but response may be more difficult to achieve than in lichen sclerosus. Far more difficult is, anyway, the control of dyspareunia, related to vestibular and vaginal erosions. These conditions always respond to some degree to the same treatment, but results are often precarious, incomplete, or, in the end, not satisfactory. Topical corticoid use in the vagina may require specific preparations, incorporating, for example, corticoid drug in adeps solidus suppositories or in carbopol gel. Topical calcineurin inhibitors may have to be tried when results are not satisfactory. Systemic oral corticoids are considered when topical treatments fail.

Surgery is sometimes necessary to lyse vaginal adhesions or to restore vestibular anatomy in case of impaired sexual function. But these conditions are difficult to treat and satisfactory results are far from being the rule.

5. Vulvar Paget's Disease

Paget's disease is mainly a disease of elderly. Mean age is around 65 years of age. A special attention should be paid to this rare disease, because its clinical appearance might suggest other conditions, such as eczema, lichen simplex chronicus, psoriasis, high grade VIN, and others. This consideration illustrates the fact that a high degree of suspicion must legitimately lead to prompt biopsy in the presence of nonspecific clinical presentations, especially if a first-line treatment fails to induce clinical improvement. On the contrary, the nonspecific presentation and complaints, in frequently old patients, explain the time between onset of complaints and diagnosis, estimated around two years [12].

Here again, pruritus is a common initial symptom. Pain can substitute when erosion occurs. Complaints are limited to the often quite delineated involved area.

Clinically, the lesion is often described as red, well-delineated, with a scaling or sometimes erosive surface, in the area of keratinized vulvar skin, and as far as perianal region (Figure 3(a)). The lesion is chronic, and a history of local discomfort may exist years before diagnosis. Early clinical signs are probably highly nonspecific, as it can be

seen in early recurrences observed during followup, where still asymptomatic patients present minor erosions or slight focal redness.

Pathology is the only reliable key for diagnosis and requires scattered large clear muciparous cells with nuclear atypia, inside the epidermis (Figure 3(b)). They can be present also in skin appendages. Underlying chronic infiltrate is often described. Presence of these cells in the dermis reflects an invasive form, which is a rare occurrence of the disease.

Etiology is related to the glandular nature of abnormal cells and to the fact that Paget's disease, either mammary or extramammary, appears nearly exclusively in skin areas where apocrine glands are present. However, some believe that malignant cells are pluripotent epidermal ones.

In spite of the fact that invasive disease is rare, association with underlying internal malignancies is a well-known characteristic of the disease, but its frequency is diversely appreciated and should be probably around 10 to 20%. For that reason, different authors recommend routine endoscopic genital-urinary and intestinal tract exploration to be performed [13].

Treatment is mainly surgical, especially when diagnosed for the first time. Unfortunately, the procedure is far from being always satisfactory. The lesion is not well delineated as the clinical presentation might suggest, and the disease is often present, somewhat far from the apparent margin. Recommended surgical margin goes up to 2 to 3 centimeters outside apparent limit of the disease, depending on whether it seems well delineated or not. Some surgeons perform frozen section margins control during surgery, but even this precaution may lead to final pathology reporting up to 10% positive margins [13]. Others reported failure rates of 20 to 60%, even if variant surgical procedures, such as Mohs micrographic surgery, improve results with recurrence rates lowered down to 16% [12].

Other treatments are currently offered and include interferon alpha, topical fluorouracil, imiquimod, CO_2 laser ablation, and, more recently, photodynamic therapy [14]. They should be considered as alternatives to surgery or to cure recurrences, while regarding radiotherapy as the ultimate solution, due to its long-term secondary effects, and taking into account the fact that any further treatment would possibly be difficult or impossible to use after radiotherapy.

Due to the nature of the disease, and its frequent microscopic extension to apparently normal surrounding skin, posttreatment biopsies are of low interest. Clinical remission and symptoms resolution are reasonable results for treatment [12].

6. Conclusion

The examples of these four frequent vulvar dermatoses underscore the importance of considering a wide range of diagnoses to obtain an accurate diagnosis. A close cooperation between clinicians and pathologists is also mandatory. Finally, exhaustive explanations relative to diagnosis and treatment and psychological support for the patient have to be integrated in the management of these diseases.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

A Methodological Evaluation of Volumetric Measurement Techniques including Three-Dimensional Imaging in Breast Surgery

H. Hoeffelin,[1] D. Jacquemin,[1] V. Defaweux,[2] and J L. Nizet[1]

[1] *Department of Plastic and Maxillofacial Surgery, CHU de Liège, Domaine Universitaire du Sart Tilman, B. 35, 4000 Liège, Belgium*
[2] *Institute of Human Anatomy, University of Liège, Domaine Universitaire du Sart Tilman, B. 35, 4000 Liège, Belgium*

Correspondence should be addressed to J L. Nizet; jlnizet@chu.ulg.ac.be

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Breast surgery currently remains very subjective and each intervention depends on the ability and experience of the operator. To date, no objective measurement of this anatomical region can codify surgery. In this light, we wanted to compare and validate a new technique for 3D scanning (LifeViz 3D) and its clinical application. We tested the use of the 3D LifeViz system (Quantificare) to perform volumetric calculations in various settings (in situ in cadaveric dissection, of control prostheses, and in clinical patients) and we compared this system to other techniques (CT scanning and Archimedes' principle) under the same conditions. We were able to identify the benefits (feasibility, safety, portability, and low patient stress) and limitations (underestimation of the in situ volume, subjectivity of contouring, and patient selection) of the LifeViz 3D system, concluding that the results are comparable with other measurement techniques. The prospects of this technology seem promising in numerous applications in clinical practice to limit the subjectivity of breast surgery.

1. Introduction

Breast reconstructive and cosmetic surgery nowadays continues to still be a subjective area where each intervention is surgeon dependent. Currently we note the unfortunate absence of measurement tools that allow objective analysis of this anatomical region, a region that is a particularly representative model of the human variability that limits its study (great variety of shapes, sizes, and compositions). Different volumetric methods of breast measurement (anthropometric formula, Grossman-Roudner method, etc.) have been described in the literature using clinical methods [1–3], casts [4], or the Archimedes principle [5, 6].

The use of medical imaging methods such as mammography [7], sonography [8], and magnetic resonance imagine (MRI) [9] has also been described. However, none of those methods have been reported to be superior to the others. More recently a French group [10] studied a method of optical scanning using structured light projection (Inspeck system) allowing them to assess the advantages and limitations of 3D imaging in breast surgery and concluding that such a volumetric calculation is perfectly suited to clinical practice.

Other groups were interested in comparing [11] different methods of volumetry (mammography, the Grossman-Roudner method, anthropometric calculation, the Archimedes principle, and casts) and demonstrated the superiority of imaging, followed by the Archimedes principle.

Lately, innovative studies [12] suggested the basis for a new biochemical imaging technology (nanodiamond imaging) that noninvasively records the distribution in two or three dimensions of biologically labeled nanodiamonds in vivo. Our study aims to evaluate and compare (using CT-scanning and the Archimedes principle) a new technique of 3D scanning acquired via stereo-visual technology (3D LifeViz) and its application to biometrics of the breast in clinical practice in terms of volume calculation and sternal notch to nipple distance.

Our study included several steps.

(i) *Experimental section*, consisting of 3 components:

(1) comparison of three volumetric techniques on anatomical prostheses with known volumes (control);
(2) comparison of three volumetric techniques on "anatomical models" (dissection and sampling from cadavers);
(3) evaluation of the LifeViz 3D camera by in situ volumetric analysis and on dissections from cadavers.

(ii) *Clinical section*, with in situ patient acquisition during consultation for cosmetic and plastic surgery for the assessment of the benefits and limitations of this 3D scanning technique.

2. Materials and Methods

2.1. LifeViz 3D System (Quantificare SA). LifeViz (Quantificare SA, 1180 Route des Dolines, Athena B, BP 40051, 06901 Sophia Antipolis, France) is a system for 3D reconstruction and analysis (Figure 1).

LifeViz is composed of a digital stereo camera, a repositioning device (we used a portable system), and 3D software: LifeViz DermaPix 3D from Quantificare and its quantification platform, LifeViz.

LifeViz has two main features for volume measurements:

(1) LifeViz "Absolute" for the evaluation of the volume in a single acquisition;
(2) LifeViz "Evolution" for the relative quantification of volume variation.

The principle of LifeViz "Absolute" is the measurement of a cavity or a protrusion using simple image acquisition. It is based on the delineation of the cavity (or protrusion) and then closing the volume with a "minimum surface," which is the mathematical equivalent of a soap film stretched across the delineated outline.

LifeViz "Evolution" is ideal for evaluation of changes in volume over time (it is well adapted to measure the boundaries of anatomical structures that are difficult to define as in the case of labial region).

2.2. Anatomical Specimens. We used nine cadavers of women aged over 60 from the Institute of Human Anatomy (CHU Liège) to measure the volume of each breast in situ and then after dissection (removal of the anatomical section).

We used four "prepared" cadavers that had undergone preparation with zinc chloride and preservatives such as ammonium. These bodies were kept in containers with the above-mentioned products for a period of 12 to 18 months (the cadavers were assigned to the Institute of Human Anatomy for medical student teaching).

Five other cadavers were "fresh" cadavers frozen at −20°C in the cold room without any preparation. We allowed them to thaw at room temperature in the Institute of Human Anatomy (15°C) for 48 hours before acquisition and dissection. These cadavers had been frozen for more than 6 months.

Figure 1: LifeViz 3D Camera.

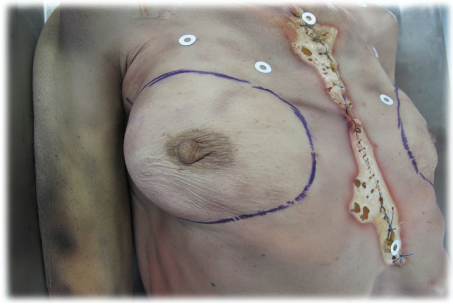

Figure 2: Contouring technique.

2.2.1. In Situ Volume Calculation from the Cadavers. First we performed a contouring that consisted of delineating the contours of the breast as closely as possible, subluxing the breast along different axes, defining the furrows to be taken as an outline, and then redrawing the contours with indelible ink. The bodies were all in the dorsal decubitus position for easy mobilization, with the upper limbs flexed to minimize the hidden parts of the breast in 3D acquisitions (to minimize the possibility of a measurement bias and therefore a truncated volume).

We took seven imaging shots in total:

(i) five views termed "stitching": front, profile 45° bilaterally, and inferior profile 45° also bilaterally, with a focal distance of 100 cm;
(ii) two views termed the "incident breast" to minimize hidden parts: inferior-lateral profile 30° bilaterally, with the same focal distance as before (Figure 2).

2.2.2. Volume Calculation on Anatomical "Samples" (Experimental). The breasts were previously marked (left/right) and then carefully resected (as contoured) avoiding taking muscle fibers from the pectoralis major muscle or the serratus anterior muscle. It should be noted that the greatest difficulty of dissection was in the frozen cadavers, where the physiological cleavage planes were almost absent and in which a more difficult but ultimately satisfactory dissection was performed.

The 3D LifeViz acquisition was performed with a single lateral view using the table since images taken perpendicularly could overestimate the density. Anatomical sections were placed on a table covered with textured stainless steel forming the surgical field. It is important to note that

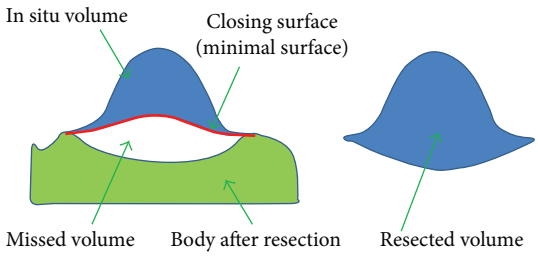

Figure 3: Schematic presentation of in situ underestimation.

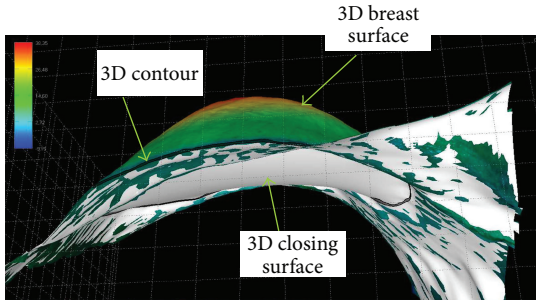

Figure 4: Reconstruction of a breast in situ with 3D LifeViz. Note the shape of the closing surface (white) below. This is a minimum area based on the 3D (black line) contour that is generally concave due to the physiological curvature of the thoracic cage.

the "absolute" measurement with the 3D LifeViz camera is accurate when the closing surface is flat, as is the case in this anatomical resection (i.e., when placed flat on the stainless steel table).

We performed the same procedure for our control measurements on prostheses of known volume (Mentor).

For the volume calculation in situ the closing surface of the 3D volume is somewhat arbitrary.

There is a minimum area in a 3D complex shape based on the 3D contour defined by the operator. Due to the physiological curvature of the chest, as well as the fact that resection will create a depression in the body, it is not expected that the in situ measurement will correspond to the measurement of a removed anatomical part (i.e., underestimation of the volume in situ) (Figures 3, 4, and 5).

2.2.3. In Situ Volume Calculation in Patient. We also undertook 3D image acquisition of 38 patients that presented for various breast-related indications at the Department of Aesthetic and Reconstructive Surgery. These patients were seen preoperatively and, if necessary, during irregular postoperative intervals. Patients were informed of the experimental nature of procedures.

The image acquisition procedure comprising seven views was the same as that used in the cadavers, specifically 5 "stitching" views and 2 "incident breast" views.

Patients stood with their back against the wall and with the posterior parts of the shoulders abutting the wall to reveal the chest in its entirety. The elbows were bent and hands were placed on the hips to minimize to the greatest extent any

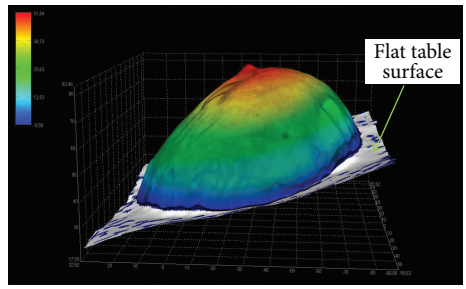

Figure 5: Reconstruction of resected breast with 3D LifeViz (anatomical part). The base is a table surface (map), where the 3D contour is quite flat, as this is also the case for the closing surface (white).

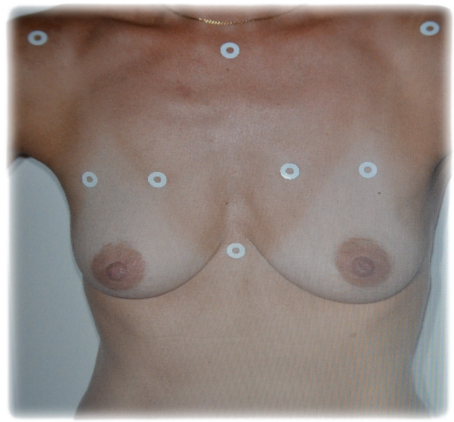

Figure 6: Anatomical spotting technique.

hidden part of the breast. Overall, this position gives a good overview of the region of interest in order to observe both breasts and their proper insertions. The position was not very comfortable for the patient, but the speed of acquisition of less than 1 minute made it tolerable.

Unable to achieve contouring in our patients during the consultation, we used marking with circular adhesive pads (which were easy to use and safe and had low cost) that were applied in relation to prominent parts of the bones (jugular notch of manubrium sterni (called the "inlet"), the xiphoid appendix, and the acromioclavicular joints bilaterally) and thus delimited the anatomical region of interest (see Figure 6). On the breast itself, the challenge was to define its upper insertion. For this we used a technique of superior subluxation according to Professor P. Blondeel at the University of Ghent (Belgium). The patient raised her breast at its base with the dorsal surface of the ipsilateral hand delimiting an upper portion taken as proximal part of insertion of the breast that we marked with our adhesive pads (Figure 6).

2.3. Archimedes' Principle. We used an old method of Archimedes' principle of buoyancy telling; "Any object, when wholly or partially immersed in a fluid, is buoyed by a force equal to the weight of the fluid displaced by the object" (the

Table 1: Data listing.

Group	Name	Side	VIZ3D_INSITU	VIZ3D_PREL	ARCHI_PREL	CTSCAN_PREL	Known vol
Patients	S1	G	205	190	220	219	—
	S1	D	200	180	200	214	—
	S2	L	485	490	550	538	—
	S2	R	380	385	450	433	—
	S3	L	280	315	450	451	—
	S3	R	320	390	480	507	—
	S4	L	70	130	150	146	—
	S4	R	180	220	250	229	—
	S5	L	330	385	425	403	—
	S5	R	285	325	400	364	—
	S6	L	105	140	180	185	—
	S6	R	135	130	170	145	—
	S7	L	125	145	200	191	—
	S7	R	170	175	250	208	—
	S8	L	470	540	600	612	—
	S8	R	510	805	1000	923	—
	S9	L	205	230	260	244	—
	S9	R	200	225	250	237	—
Controls	T1		—	75	55	60	60
	T2		—	157	150	146	150
	T3		—	339	350	335	330
	T4		—	461	450	474	460

volume is equal to the immersed volume of the body). This method is attractive because of its simplicity, reproducibility, low cost, and speed of execution. For this we used a Brand measuring cup of 3000 mL/50 mL by graduation. Then each freshly collected anatomical resected breast was immersed in a determined volume of liquid, in this case 2000 mL of water (the volume was the same for all measures). After recording the different displaced fluid values, we satisfactorily calculated the various results. We repeated the maneuver for the 18 breasts (cadaveric) as well as for four anatomic prostheses of known volume.

2.4. CT-Scan. For scan acquisitions we used a GE Bright Speed 16 strips, model 2010. For this purpose we used the volumetric software "Paint on Slice," an application of segmentation program "Advantage Work Station GE 4.6" (basic volume viewer). We undertook volume acquisition in spiral mode (abdomen program) without injection of contrast, in axial sections with cuts of 1.25 mm (thickness) every 1 mm (interval), and with 120 kV for 209 mAs per procedure. The acquisition time was 20 seconds for the first series (anatomical parts) and 13.83 sec for the second series (prostheses).

3. Results

3.1. Data Handling and Statistics. We measured the volume (cm^3) of the left and right breasts of nine cadavers (S1–S9) using our various techniques. The VIZ3D technique used a 3D camera for volume calculation. This technique was performed once in situ (VIZ3D_INSITU) and once on the resected sample (VIZ3D_PREL). The other two techniques were performed only on the resected sample, namely, Archimedes' manual method (ARCHI_PREL) and the CT scan (CTSCAN_PREL).

The volume was also measured by the 3 techniques (VIZ3D_PREL, ARCHI_PREL, and CTSCAN_PREL) in 4 breast implants with different sizes termed controls of known volume (VOLCONNU).

We performed measurements in triplicate on the cadaver or anatomical part, the prosthesis or in vivo, and calculated the mean volume measurement for each method, rounded up to the closest unit. We tested three types of 3D LifeViz cameras that differed only by the focal distance of acquisition of 70, 80, and 100 cm. After testing, we kept the camera focal length of 100 cm to allow for optimal breast measurement in our study.

3.2. Data Listing (Table 1)

3.2.1. Methodology. To assess concordance between two series of measurements (technique 1 and technique 2) several statistical methods can be used.

Coefficient of Intraclass Correlation (ICC). The ICC is a measure of concordance between two series where the studied variable is continuous (the volume measures in cm^3). The closer the ICC is to 1, the better the concordance is between

Table 2: VIZ3D in situ versus VIZ3D sampling ("naive" operator).

(a)

Name	Side	VIZ3D_INSITU	VIZ3D_SAMP	dVIZisVISp
S1	G	205	190	15
S1	D	200	180	20
S2	L	485	490	−5
S2	R	380	385	−5
S3	L	280	315	−35
S3	R	320	390	−70
S4	L	70	130	−60
S4	R	180	220	−40
S5	L	330	385	−55
S5	R	285	325	−40
S6	L	105	140	−35
S6	R	135	130	5
S7	L	125	145	−20
S7	R	170	175	−5
S8	L	470	540	−70
S8	R	510	805	−295
S9	L	205	230	−25
S9	R	200	225	−25
	Mean ± SD	258.6 ± 133.5	300.0 ± 178.0	−41.4 ± 68.9
	Median			−30
	P25–P75			−55–−5
	SD (robust)			37.0

(b)

Method 1	Method 2	N	ICC (ICC*)	Difference	Paired Student's t-test P value	Wilcoxon P value	CV (%)	Bland-Altman r (P value)
VIZ3D in situ	VIZ3D sample	18	0.878 (0.698)	−41.4 ± 68.9	0.021	0.0008	19.9	Pearson: −0.66 (0.0031) Spearman: −0.39 (0.10)

There was a significant difference of 41.4 ± 68.9 cm^3 ($P = 0.021$) between two methods. The in situ method underestimates the volume compared to the resection-based method.

the two series. Zero (ICC = 0) signifies the absence of concordance.

Paired Samples Student's t-test/Wilcoxon Signed Rank Test. The paired samples Student's t-test is used to test the hypothesis that the average difference between two measures (techniques) is absent. A P value is associated with value obtained from Student's t-test. If the test is rejected ($P < 0.05$), that means there is a systematic difference between the values provided by two techniques. Otherwise, we consider that the means in two techniques give the same values. Wilcoxon test of signed ranks is a nonparametric test corresponding to the Student's t-test and compares the medians.

Reproducibility of Measurements (CV). To measure the reproducibility of a test, namely, the ability to reproduce the same value by repeating the measurement, we can use two results obtained for each sample as if they had both been obtained using the same technique. Reproducibility is best expressed by a coefficient of variation CV (%). The procedure is as follows: consider n pairs of measurements $\{(x_{i1}, x_{i2}), i = 1, \ldots, N\}$. Note that $\overline{x} = (\overline{x}_1 + \overline{x}_2)/2$ and $s^2 = (\sum d_i^2)/(2n)$, where $\overline{x}$ is the mean of means of two measurements and $\{d_i = x_{i1} - x_{i2}, i = 1, \ldots, N\}$. So, CV $= (s/\overline{x}) \times 100\%$. The lower the CV is, the better the reproducibility is.

Bland-Altman Plot. In a Bland-Altman plot, for each pair of measurements $\{(x_{i1}, x_{i2}), i = 1, \ldots, N\}$ we report on the abscissa the mean of two measurements $\overline{x}_i = (x_{i1} + x_{i2})/2$ and on the ordinal the difference $d_i = x_{i1} - x_{i2}$. In principle, there should not be any association between d_i and $\overline{x}_i$. We can test this hypothesis by calculating the correlation coefficient r (classical or spearman) between the two series and associate it to the P value. The absence of correlation indicates that the gap between two measurements does not vary with volume.

3.3. Cadavers: Comparison of VIZ3D In Situ versus VIZ3D Sampling (Table 2). There was a significant difference of 41.4 ± 68.9 cm^3 ($P = 0.021$) between two methods. The in situ method underestimates the volume compared to the

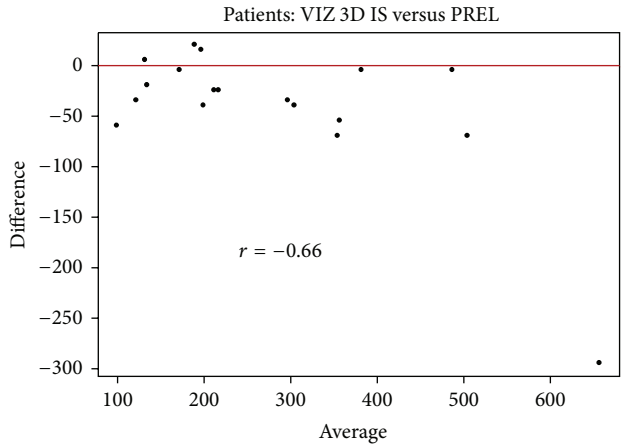

Figure 7: Bland-Altman plot comparing VIZ3D in situ versus VIZ3D sample from the bodies. This graph shows stability of measures; the difference between the measurements does not depend on volume.

resection-based method. The concordance between the two methods is good (0.87) but the lower confidence limit is not very high. The difference between two methods is 19.9% (Figure 7).

Regarding the underestimation of in situ measurements, additional information could be assessed. The 3D image could be obtained after resection of the anatomical area. This would allow measurement by the 3D LifeViz camera of the hollow anatomical section left by the resection. This would allow one to compare the differences in volume before and after resection, the volume of the resected portion, which would explain the underestimation of the volume due to the hollow left by resection.

For its part, the company trained operator also made measurements of the same areas as us. Note that all their measurements were made with a single view of the 3D camera (instead of 5 + 2 made by us), with the angle that seemed the most appropriate. This was possible in all subjects except one case in situ and in one localization for this patient, where the arm limited this measurement. As a result, the company excluded this particular image of the in situ experiment and therefore used 8 subjects instead of 9 in our series. Their number 7 has only one image on the right side because the left side contained too many hidden regions and therefore could bias the measurement. They did not perform the mean of three measurements (rather, one single measurement in the optimal orientation of imaging was used). Their trained experience in shooting and contouring (one large contour will permit better definition of the closing surface without increasing the measured total volume and has less risk of losing a part of the volume of the object) as compared to "naive" operators is predictable. Their findings after statistical integration are shown. Note that the order of their subjects is not identical to ours (Table 3).

We observed a significant difference of $47.1 \pm 36.5\,\text{cm}^3$ ($P = 0.0002$) between the two methods. The concordance between the two methods is very good (0.92) but the lower confidence limit is not particularly high. The coefficient of variation between two methods is 14.5%. As expected, the company measurements are more precise and better correlated than ours. This indicated that the measurements are highly operator dependent. Therefore for future clinical use by various practitioners standardized training by the company itself is needed to have reproducibility of measurements (particularly important in clinical follow-up or pre- and postoperative comparison).

3.4. Controls: Comparison of Methods (Table 4). We used as "controls" four anatomic Mentor implants with known volume (T1: 60cc, T2: 150cc, T3: 330cc, and T4: 460cc). Using these controls we compared one with another using our three methods of study and we found good concordances and relatively low coefficients of variation. However, the results should be assessed with caution because there were only four observations (Figure 8).

3.5. Cadavers: Comparison of Methods Using Resected Samples. In the cadavers (S1–S9), when comparing the volume with the reference method (here it was the CT scan), there was a mean difference of $47.2 \pm 39.0\,\text{cm}^3$ for VIZ3D method and a mean difference of $13.1 \pm 23.6\,\text{cm}^3$ for the Archimedes method. In all cases, the difference was statistically significant. The best concordance with the CT scan was obtained with the Archimedes method and with this the ICC was 0.992 and the lower confidence limit was very high (0.978). For the VIZ3D method, we obtained a concordance of 0.951 (0.629) between the samples. For the VIZ3D method, the ICC was high but the lower confidence limit was below that observed for the Archimedes method. The coefficient of variation for the Archimedes method was also lower (5.29%) as compared to the VIZ3D technique (in situ 29.9% and on samples 13.2%).

With these results, we can suggest that imaging is the technique of choice, followed by the Archimedes principle and then the 3D imaging LifeViz.

If we look at the overall concordance between three methods, we obtain an ICC of 0.96 (inferior limit = 0.90), suggesting comparability of these three methods (Figures 9, 10, 11, and 12).

3.6. Acquisition of Patients' Images. Over a period of a month we scanned 38 patients, preoperatively in the majority of cases. These scans allowed us to identify issues related to the acquisition of patients in clinical practice (during consultation) and to gain the necessary experience with a view to clinical use. Indications for surgery in patients who received at least one 3D acquisition were varied, such as, breast reconstruction by prosthesis and/or lipomodelling, remodeling of breast reconstruction, breast asymmetry, breast ptosis, or breast reduction.

4. Discussion

This study evaluates the application of 3D image acquisition to breast surgery using a new technique for 3D stereovision, LifeViz. We demonstrated a good correlation between the

Table 3: VIZ3D in situ versus VIZ3D sampling ("trained" operator).

(a)

Subject	Side	Volume_in_situ	Volume_resection	diff
P001	Right	384.5	424.7	−40.2
P001	Left	507.7	520.8	−13.1
P002	Right	362.3	436.8	−74.5
P002	Left	276.0	402.3	−126.3
P003	Right	171.5	234.0	−62.5
P003	Left	69.1	156.7	−87.6
P004	Right	285.8	350.1	−64.3
P004	Left	345.1	398.5	−53.4
P005	Right	153.2	133.2	20.0
P005	Left	108.3	173.0	−64.7
P006	Right	168.9	205.0	−36.1
P006	Left	123.8	165.4	−41.6
P007	Right	525.7	574.9	−49.2
P008	Right	244.7	248.2	−3.5
P008	Left	230.3	240.3	−10.0
	Mean ± SD	264 ± 140	311 ± 142	−47.1 ± 36.5
	Median	245	248	−49.2
	P25–P75	153–362	173–425	−64.7–−13.1
	SD (robust)	155	186	38.2

(b)

Method 1	Method 2	N	ICC (ICC*)	Difference	Paired Student's t-test P value	Wilcoxon P value	CV (%)	Bland-Altman r (P value)
In situ	Resection	15	0.92 (0.42)	−47.1 ± 36.5	0.0002	0.0004	14.5	Pearson: $r = -0.06$ ($P = 0.82$) Spearman: $r = 0.04$ ($P = 0.89$)

We observed a significant difference 47.1 ± 36.5 cm^3 ($P = 0.0002$) between the two methods. The concordance between the two methods is very good (0.92) but the lower confidence limit is not particularly high.

Table 4: Comparison of methods (controls).

Method 1	Method 2	N	ICC (ICC*)	Difference	Student's t-test P value	Wilcoxon P value	CV (%)	Bland-Altman r (P value)
T1–T4								
Known volume	VIZ3D samp.	4	0.999 (0.968)	−8.00 ± 5.77	0.070	0.13	2.63	Pearson: 0.84 (0.16) Spearman: 0.80 (0.20)
Known volume	Archimedes' samp.	4	0.999 (0.982)	−1.25 ± 13.1	0.86	0.99	3.23	Pearson: −0.10 (0.90) Spearman: 0.20 (0.80)
Known volume	CT scan samp.	4	0.999 (0.994)	−3.75 ± 7.76	0.41	0.50	2.16	Pearson: −0.899 (0.10) Spearman: −0.80 (0.20)
VIZ3D samp.	Archimedes' samp.	4	0.997 (0.982)	6.75 ± 13.0	0.38	0.50	3.65	Pearson:−0.47 (0.53) Spearman: −0.40 (0.60)
VIZ3D samp.	CT scan samp	4	0.998 (0.986)	4.25 ± 12.4	0.54	0.63	3.18	Pearson: −0.956 (0.044) Spearman: −1.00 (<0.0001)
Archimedes' samp.	CT scan samp	4	0.997 (0.973)	−2.50 ± 16.5	0.78	0.88	4.06	Pearson: −0.34 (0.66) Spearman: −0.20 (0.80)

Using these controls we compared one with another using our three methods of study and we found good concordances and relatively low coefficients of variation.

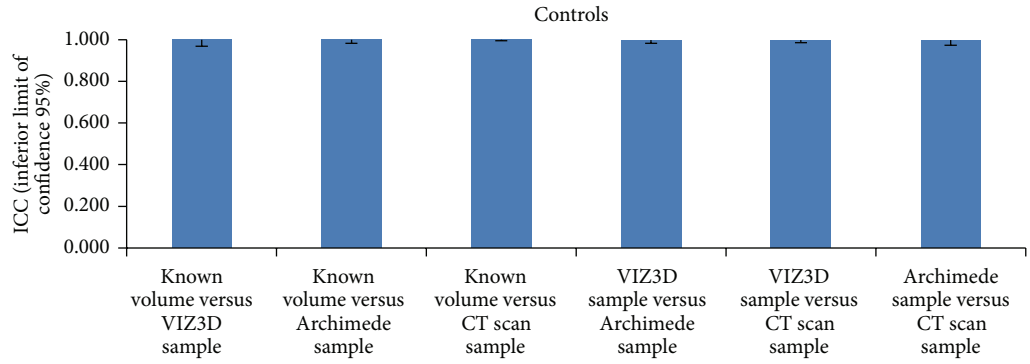

Figure 8: Comparisons of methods (controls) based on ICC values. The results should be assessed with caution because there were only four observations.

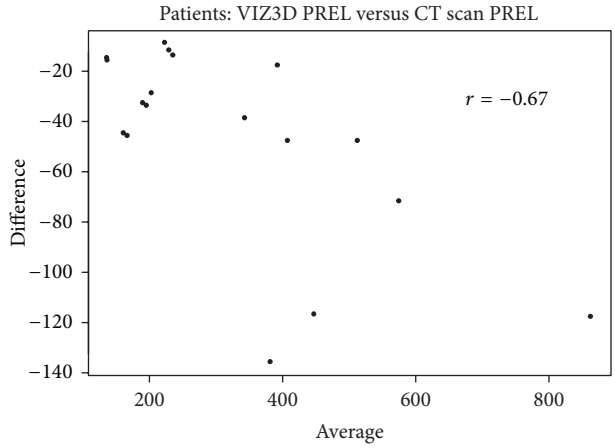

Figure 9: Bland-Altman plot comparing VIZ3D sampling versus CT scan sampling (parts of dissection). When comparing the volume with the reference method (here it was the CT scan), there was a mean difference of 47.2 ± 39.0 cm^3 for VIZ3D method.

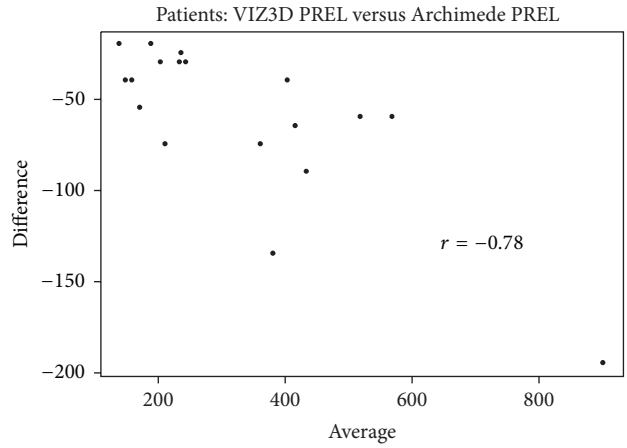

Figure 10: Bland-Altman plot comparing VIZ3D sampling versus Archimedes' method on samples (anatomical dissection). When comparing the volume with the reference method (here it was the CT scan), there was a mean difference of 13.1 ± 23.6 cm^3 for the Archimedes method. In all cases, the difference was statistically significant.

measurements made using the CT scan and Archimedes' principle buoyancy with anatomical samples and isolated implants and with the 3D camera. However, the volume calculation in situ (cadaveric and clinical) needs to be improved (due to significant underestimation of volume).

The advantages and limitations of this new technology should be considered.

Advantages. (i) Portable device: this is a major advantage of this system that could increase clinical use (the transportation to consultation from one department to another), usage in operating rooms (acquisition could be performed pre-, per, and immediately postoperatively or later), mobility (low weight and acceptable size), and usage in any place (no need of a "special" room). We would emphasize the substantial autonomy for several days of use.

(ii) Safety: no irradiation, a flash similar to those used in photographic cameras and harmless to the human eye, pain-free technique.

(iii) Low stress for the patient: very short acquisition time (of the order of seconds), which increases the quality of measurements by avoiding patient movement (that can produce image distortion). The acquisition can be performed sitting or standing. Here we chose the standing position with hands on hips (intermediate position between the anatomical position and the raised arms position) to minimize the hidden parts of the chest and to maintain the advantages of both positions above (this was possible because our patients were all preoperative); the intermediate position was originally described by Sinna et al. [10].

(iv) Additional value: the advantages over traditional photography (the objective forms angles of view not accessible by standard simple photography).

(v) Cost: <15000 Euros for the entire apparatus.

(vi) Noninvasive technique: noncontact nature of the process (therefore possible to use even on sensitive soft tissue, and it doesn't cause any deformation).

Disadvantages. (i) A problem is that the focal length needs to be respected (between 80 and 120 cm), with the risk of image

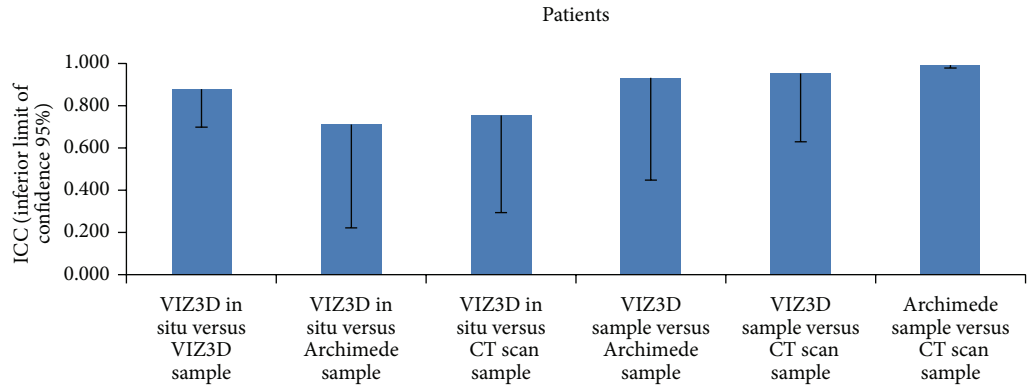

Figure 11: Comparison of methods (cadavers) based on ICC values. The best concordance with the CT scan was obtained with the Archimedes method and with this the ICC was 0.992 and the lower confidence limit was very high (0.978). For the VIZ3D method, we obtained a concordance of 0.951 (0.629) between the samples. For the VIZ3D method, the ICC was high but the lower confidence limit was below that observed for the Archimedes method.

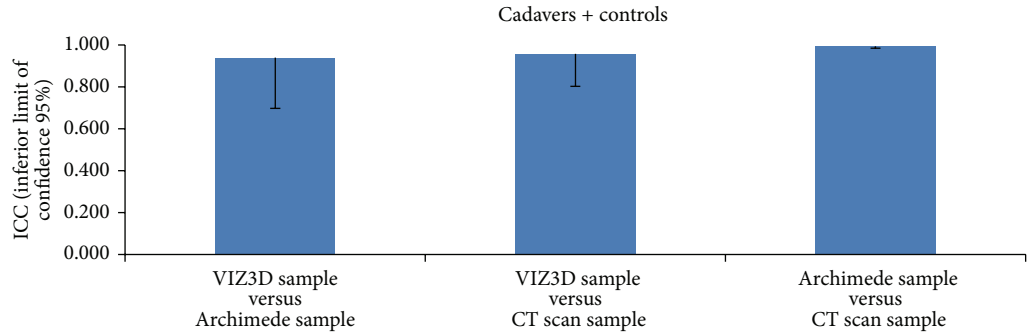

Figure 12: Comparison of methods (cadavers + controls) based on ICC values. If we look at the overall concordance between three methods, we obtain an ICC of 0.96 (inferior limit = 0.90), suggesting comparability of these three methods.

distortion as a penalty (computer contouring difficulties occur in these conditions) and the problem of comparability of results.

(ii) Difficulty in defining breast contours (upper limit is the axillary pillar).

(iii) Underestimation of the volume in situ, hence the difficulty of reliable clinical utilization.

(iv) Selection of patients: patients with significant ptosis or who are significantly overweight disrupt the scans, resulting in the measurement bias by increasing the hidden regions (in particular that of segment III) but also making the breast limits barely perceptible during contouring.

(v) Subjectivity in contouring: contouring is very operator dependent, which makes it difficult to reproduce.

5. Conclusion

This technology appears to offer a promising future because of its multiple applications particularly in clinical practice. The technology challenges the subjectivity of surgery, allowing the more likely obtainment of predictable and defined results, improving patient satisfaction and serving as an objective and reliable measurement tool for the practitioner to improve the quality of interventions and outcomes. In our evaluation of the 3D LifeViz camera, in situ volume calculations alone remain perfect for true routine clinical use.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the content of this paper.

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Review Article

Psoriasis: Female Skin Changes in Various Hormonal Stages throughout Life—Puberty, Pregnancy, and Menopause

Romana Ceovic,[1] Marko Mance,[2] Zrinka Bukvic Mokos,[1] Maja Svetec,[3] Kresimir Kostovic,[1] and Daska Stulhofer Buzina[1]

[1] *Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, 10 000 Zagreb, Croatia*
[2] *Ministry of Justice, 10 000 Zagreb, Croatia*
[3] *Institute of Emergency Medicine, 42 000 Varazdin County, Croatia*

Correspondence should be addressed to Romana Ceovic; romana.ceovic@zg.htnet.hr

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Psoriasis is one of the most prevalent immune mediated skin diseases worldwide. Despite the large prevalence in both men and women, the pathogenesis of this disease has not yet been fully clarified. Nowadays, it is believed that psoriasis is most likely a T helper Th1/Th17 induced inflammatory disease. Stressful life situations are known to cause flare-ups and psoriasis activity may be linked to stress from major life events. We know that stress greatly affects both the hormone and immune systems and that there are many different hormonal phases throughout a woman's lifetime. The severity of psoriasis may fluctuate or be influenced by each phase and this relationship can be seen as disease frequency seems to peak during puberty, postpartum, and menopause when hormone levels fall, while symptoms improve during pregnancy, a state when hormone levels are increased.

1. Introduction

Psoriasis affects approximately 25 million people in North America and Europe and is one of the most prevalent immune mediated skin diseases in adults [1]. It is a chronic, inflammatory skin disorder characterized by erythematous, scaly patches, and plaques that can affect any part of the body [2]. The incidence of psoriasis is similar in male and female populations, with the mean age of presentation in females at 26.94 ± 14.94 years [3].

The pathogenesis of psoriasis is considered to be an immune mediated process that takes place upon a favorable genetic background. The presence of a yet unknown (auto)antigen causes the generation of effector T-cells that infiltrate the skin and initiate the inflammatory process [4]. The disease pathogenesis is linked to many interactive responses among infiltrating leukocytes, resident skin cells, and an array of proinflammatory cytokines, chemokines, and chemical mediators produced in the skin [5]. Nowadays, it is believed that psoriasis is most likely a T helper Th1/Th17 induced inflammatory disease [6].

Psoriasis is associated with metabolic syndrome and the association increases with increasing disease severity [7]. The impact of psoriasis on the patient quality of life is similar to that in patients living with insulin-dependent diabetes, depression, and cardiovascular diseases [8]. Various environmental risk factors, including trauma to the skin, infections, obesity, smoking, alcohol use, emotional stress, and various drugs, have been associated with psoriasis. Disease flare-ups are known to occur in stressful life situations and certain literature links psoriasis activity to stress from major life events [9]. The exact mechanism of how psoriasis is induced or aggravated is not known, but we do know that stress greatly affects both the hormone and immune systems [10, 11]. There are many different hormonal phases throughout a woman's lifetime and the first symptoms of psoriasis can be seen at any time, that is, in childhood, menarche, pregnancy, during hormonal contraceptive use, and menopause [12]. The severity of psoriasis may fluctuate or be influenced by each phase and this relationship can be seen as disease frequency seems to peak during puberty, postpartum, and menopause, when hormone levels fall, while symptoms improve during pregnancy,

a state when hormone levels are increased [13, 14]. Recent studies have shown that female hormones significantly affect the biological and immune changes in the skin [15]. The Th2 immune response, shown to be potentiated by estrogen, results in increased concentrations of interleukin (IL)-4, IL-5, and IL-10, while androgens have been found to increase the Th1 response leading to increased IL-2 production and activation of CD8+ T-cells. Interestingly, these differences are marked in reproductive years but disappear after menopause [16].

2. Psoriasis during Puberty

Psoriasis in childhood and adolescence is not uncommon and many studies indicate the appearance of psoriatic lesions by 16 years of age in one-third of patients [17]. It has been observed that 63.5% of patients develop the disease before the age of 30 [18]. The age at onset was documented by Swanbeck et al. to begin around puberty as well as between the ages of 30 and 50 [19]. This bimodal age at onset has also been described by Henseler and Christophers, who report on the mean age at onset of psoriasis presentation to range between 15 and 20 years of age in 2,147 patients, with a second peak occurring at the ages of 55–60 [20]. A relationship between psoriasis and hormonal changes in different stages of life has been observed; however, it has not yet been identified [21]. In women, hormonal changes such as those that occur at puberty can trigger or worsen psoriasis, which has also been mentioned by Islam et al. [3, 22]. Murase et al. also observed a relationship between the development of psoriasis and puberty and found that many people developed their first psoriatic lesions just after puberty, which correlates with the decrease in hormone levels [13]. A recent study by Kanda and Watanabe has revealed that sex hormones manifest a variety of biological and immune effects in the skin and that hormone fluctuations during a woman's menstrual cycle can affect psoriasis [15]. During menstrual cycle, the follicle within the ovary is actively secreting estrogens until their serum levels reach the threshold value. After approximately 10 days, corpus luteum begins to degenerate, with estrogen and progesterone concentrations declining at around day 26 of the cycle. Luteinizing hormone (LH) level begins to rise and the follicles are therefore stimulated to mature, so that by the beginning of the new cycle, estrogen levels are once again on the rise [21]. Kanda and Watanabe have found that menstruation is associated with modulation of the natural course of psoriasis, suggesting that skin inflammation may be hormone induced. It has been observed that estrogen downregulates the production of the neutrophil, T-cells, and macrophage-attracting chemokines, CXCL8, CXCL10, and CCL5, by keratinocytes, and suppresses IL-12 production and antigen-presenting capacity while enhancing anti-inflammatory IL-10 production by dendritic cells, indicating how inflammation in psoriatic lesions may be linked to estrogens [15].

Increased levels of sex hormones, in particular estrogens, which are known to promote keratinocyte proliferation via specific receptor-mediated mechanisms, may explain the perimenarchal increase in the prevalence of psoriasis [23–28]. This mechanism appears to be significant in the wound-healing process, suggesting that this effect alone may provide a significant stimulus to the development of epidermal hypertrophy characteristic of psoriasis [29, 30].

Sex hormones are also known to influence inflammation [15, 31]. The increased levels of estrogen at menarche may influence the Th1 and Th2 immune responses through cytokines and chemokines, including monocyte chemoattractant protein-1 (MCP-1) production [23, 32, 33]. These changes may stimulate both the cellular activity and tumor necrosis factor (TNF)-alpha-induced inflammatory response, potentially providing a more direct link to the pathophysiology of psoriasis [23, 34, 35].

Generally, it can be assumed that high levels of estrogens seem to have a rather regulatory and inhibiting effect on many components of the immune response, while low levels can be stimulating [32, 36, 37]. These various regulatory effects of sex steroids and their fluctuations during puberty and adolescence have been linked to many skin conditions including psoriasis and are the focus of many therapeutic or prophylactic measures [15]. It is important for the patient as well as the physician to realize that psoriasis is a chronic condition and that hormonal changes can influence the course of the disease. Children, parents, and teenagers need to realize that psoriasis may recur throughout the patient's life and that it is of utmost importance to understand the disease and the hormonal phases that may aggravate it [22].

3. Pregnancy

Psoriasis affects women of all ages including reproductive years [38]. Estrogen and progesterone levels steadily increase throughout pregnancy until antepartum period [19]. Mowad et al. report on patients taking high-dose estrogen oral contraceptives and showing general improvement of their psoriasis, whereas Vessy et al. in their study in 92 patients concluded that there was little evidence for any effect of oral contraceptive use on psoriasis [39, 40]. Boyd and King found a different correlation when observing a patient whose psoriasis responded favorably to the administration of the antiestrogen compound tamoxifen [41].

During a woman's reproductive years, stressful life events such as pregnancy characterized by multiple physiologic changes influence the development of psoriasis and affect its clinical expression [3, 42]. Sex hormones, especially estrogen and prolactin (PRL), have an important role in modulating the immune response. Prolactin secreted from the pituitary gland as well as other organs and cells has an immune stimulatory effect and promotes autoimmunity. It interferes with B-cell tolerance induction, enhances proliferative response to antigens and mitogens, and increases the production of immune globulins, cytokines, and autoantibodies. Patients with hyperprolactinemia (HPRL) present with many different clinical manifestations, one of them being psoriasis. There are data indicating a correlation between PRL levels and disease activity [43]. Dhabhar has documented a connection between stress-related neurotransmitters, hormones, and other factors

and exacerbation of certain immunopathologic conditions such as psoriasis [44]. Enhanced vascular endothelial growth factor (VEGF) production in macrophages is stimulated by estrogen, an effect that is antagonized by androgens, and it is believed that imbalance in hormone ratios could be related to the development of dermatologic diseases during pregnancy [13, 15, 44]. Oumeish and Al-Fouzan recognized the potential role of sex hormones in the etiology of psoriasis, since pregnancy, a state of natural immunomodulation, is associated with alleviation or exacerbation in various inflammatory diseases, including psoriasis [45, 46].

In their study, comparing hormonal effect on psoriasis in pregnancy, Murase et al. report that, during pregnancy, 55% of patients experienced improvement, 21% no change, and 23% worsening, while, postpartum, only 9% of patients experienced improvement, 26% no change, and 65% worsening. They found that psoriatic body surface area (BSA) significantly decreased from the 10th to the 20th week of gestation ($P < 0.001$) when compared with controls, whereas BSA significantly increased by 6 weeks postpartum ($P = 0.001$). Furthermore, in patients with 10% or greater psoriatic BSA, who reported improvement ($n = 16$; mean BSA, 40%), lesions decreased by 83.8% during pregnancy. They found that there were significant or near significant correlations between improvement in BSA and concentrations of certain hormones such as estradiol ($P = 0.009$; $r = 0.648$), estriol ($P = 0.06$; $r = 0.491$), and the estrogen to progesterone ratio ($P = 0.006$; $r = 0.671$). Therefore, it was concluded that high levels of estrogen correlated with an improvement in psoriasis, whereas progesterone levels did not correlate with psoriatic change [13]. Many studies investigated the relationship between hormones and psoriasis; it has been observed that worsening of symptoms occurs when estrogen and progesterone levels drop postpartum, prior to menses, and at menopause, while most patients receiving hormone therapy around menopause noted no change in their condition [39, 47–50].

Carlsten et al. have described how pregnancy and hormonal changes lead to improvement of psoriasis; they propose that estrogens have both an immunosuppressive and immunostimulatory property promoting a state of immune tolerance [51]. Estrogens have been shown to stimulate B-cell mediated immunity while suppressing T-cell mediated immunity; progesterone, being primarily immunosuppressive, downregulates the T-cell proliferative response and has been shown to be the key factor in immunosuppression [51–56]. Therefore, it has been hypothesized that high levels of progesterone would correlate with improvement of psoriatic symptoms [46]. It was observed that progesterone levels increased more dramatically during pregnancy compared with estrogen levels and it has been proposed by Carlsten et al. that the change in the estrogen-progesterone ratio produces an altered immunity [39, 51]. However, Murase et al. report on findings indicating that increased estrogen levels and especially increased levels of estrogen relative to progesterone correlate with improvement of psoriasis. They found that progesterone levels alone did not correlate with change in psoriasis and therefore it can be assumed that patients who experience an improvement of psoriasis have higher levels of estrogen relative to progesterone during pregnancy, whereas those who have lower ratio levels will remain unchanged or potentially worsen [13]. The estrogen concentration in peripheral blood gradually increases throughout the early to late stages of pregnancy, subsequently decreasing after parturition and eventually reaching nonpregnancy group levels within one month postpartum [57]. Weatherhead et al. found that half of their patients with psoriasis presented with a flare-up of symptoms within six weeks of delivery, which correlates with the previously mentioned observations implicating the role of hormones in psoriatic symptoms [58].

Dermatological changes occur in about 90% of pregnant women in one form or another and the associated skin changes may be either physiological (hormonal), changes in preexisting skin diseases, or development of new pregnancy-specific dermatoses. All of these conditions can be linked to either profound hormonal, vascular, metabolic, or immune changes occurring during pregnancy and treatment can be difficult [59]. Unfortunately, treating psoriasis in pregnant and lactating women presents a special challenge. Due to obvious ethical reasons, prospective randomized control trials have not been conducted in this patient population, although these patients do encounter new-onset psoriasis in addition to flares and may require treatment throughout their pregnancies [60].

4. Menopause

During menopause, endocrine disorders can be the cause of many skin diseases or conditions. Menopause, like pregnancy or menstruation, modulates the natural course of psoriasis [15, 61]. In perimenopause, many different hormonal changes occur and the onset of perimenopause or menopausal transition is marked by the end of menstrual cycle regularity and is associated with decreases in the production of ovarian inhibiting hormones [62]. During the early follicular phase of the menstrual cycle, slightly elevated but highly fluctuating follicle-stimulating hormone (FSH) levels may be observed. These levels gradually become consistently elevated into the late perimenopause and postmenopause, while estrogen and progesterone levels decrease and luteinizing hormone levels increase as the woman approaches menopause. The postmenopausal period is divided into early and late phases, marked by significant decreases in estrogen production, an overall state of hypogonadism, stability in the hypothalamic-pituitary-gonadal axis, and elevated FSH levels [63]. A decrease in estrogen during menopause is believed to be a major factor in the occurrence or exacerbation of psoriasis flare-ups in patients already suffering from psoriasis and it is believed that reduced estrogen levels lead to insufficient Th1 cell-mediated response inhibition, playing a major role in the pathogenesis of psoriasis. The study by Kanda and Watanabe has shown that β17 estradiol (E2) inhibits the production of IL-12 and TNF-α, reducing the ability of dendritic cells to present antigens and therefore to stimulate the synthesis of the anti-inflammatory cytokine IL-10 by T-lymphocytes, and also exhibits an inhibitory effect on the Th1-type immune response. Therefore, a fall in estrogen concentration in

postmenopausal women can be attributed to the exacerbation of psoriasis. This finding may be useful since estrogen and/or progesterone may be potentially beneficial in the treatment of psoriasis [15]. In a study conducted by Mowad et al., menopausal women had an exacerbation of psoriasis in 48% of cases, while only 2% showed improvement; 27% of those observed noticed a link between psoriasis and hormonal changes [39]. Swanbeck et al. report on similar results and conclude that the late onset of psoriasis is more common in women than in men, suggesting that hormonal changes associated with menopause are a potential factor contributing to the development of psoriasis [64].

Generally, it can be assumed that high levels of estrogen have a rather regulatory and inhibitory effect on many components of the immune response, while low levels can affect it or even be stimulating [32, 36, 37]. A good example is the TNF-α molecule; the low estrogen concentrations typical of postmenopausal women have a stimulatory effect on the production of this cytokine, whereas high concentrations inhibit its synthesis, which could be crucial in the understanding of psoriasis in postmenopausal women [16, 32]. Therefore, it can be assumed that the decline in estrogen levels during menopause may be responsible for worsening of psoriasis [32].

5. Conclusion

The severity of psoriasis in a female patient may fluctuate with hormonal changes since psoriasis develops more frequently or gets worse at puberty, with another smaller peak at menopause. Often, there is a marked symptomatic improvement or even disappearance during pregnancy, only to reappear after childbirth. With such strong data linking hormones and psoriasis, estrogen and/or progesterone may be potentially useful in the treatment of psoriasis. Due to ethical reasons, clinical trials are not conducted in pregnant patients, although this population does encounter new-onset psoriasis in addition to disease flare-ups. Additional research is required before any conclusions can be drawn. It is important for the patient as well as the physician to be aware of the possible relationship between psoriasis and the hormonal phases throughout a woman's lifetime in order to effectively control or ease any symptoms that may arise.

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Clinical Study

The Weather-Beaten Dorsal Hand Clinical Rating, Shadow Casting Optical Profilometry, and Skin Capacitance Mapping

Marie Delvenne,[1] Claudine Piérard-Franchimont,[1] Laurence Seidel,[2] Adelin Albert,[2] and Gérald E. Piérard[1,3]

[1] *Laboratory of Skin Bioengineering and Imaging (LABIC), Department of Clinical Sciences, Liège University, 4000 Liège, Belgium*
[2] *Department of Medical Informatics and Biostatistics, University of Liège, 4000 Liège, Belgium*
[3] *Department of Dermatology, University Hospital St Jacques, 25030 Besançon, France*

Correspondence should be addressed to Gérald E. Piérard; gerald.pierard@ulg.ac.be

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Laypeople commonly perceive some skin xerosis and withering (roughness) changes during winter on some parts of the body, particularly on the dorsal hands. The aim of the study was to assess the withered skin surface changes occurring during the four seasons. A total of 47 menopausal women completed the study. A group of 31 volunteers were on hormone replacement therapy (HRT) and 16 were out of HRT. Skin xerosis and scaliness were rated clinically. In addition, skin whitening was assessed by computerized shadow casting optical profilometry and by skin capacitance mapping. The volunteers were not using topical creams and over-the-counter products on their hands. Marked changes, recorded over the successive seasons, corresponded to patchy heterogeneous stratum corneum hydration and heterogeneous skin surface roughness changing over seasons; they likely resulted from changes in the environmental temperature and atmosphere moisture. The severity of the changes revealed by clinical inspection was not supported by similar directions of fluctuations in the instrumental assessments. This seemingly contradiction was in fact due to different levels of scale observation. The clinical centimetric scale and the instrumental inframillimetric scale possibly provide distinct aspects of a given biological impact.

1. Introduction

Seasonal variations in environmental conditions are prone to alter the skin presentation particularly on the legs, face, and back of the hands [1, 2]. For laypeople, the resulting aspect and feel are described as a dry and rough skin. Various clinical scales have been designed for rating the xerotic harsh conditions [3].

The current overwhelming trend steering dermatology aims toward making the descriptions more scientific and clearly identifiable. The skin microrelief and its seasonal withered aspect are conveniently assessed using a series of dedicated noninvasive and scientifically validated methods [4–6]. One of these relies on the collection of negative replicas from the skin surface. The microrelief profile is then conveniently quantified using shadow casting optical profilometry (SCOP). The SCOP procedure for skin analysis consists of lighting the sample by a parallel light source with a defined incident angle. The microrelief generates shadows which are wider when skin peaks and crests are taller [7–9]. The process averages the scannings of a series of parallel lines of 2D assessments.

Both the relative moisturization of the upper stratum corneum (SC) and the pattern of the skin microrelief are conveniently recorded by skin capacitance mapping/imaging (SCMI). The method was previously described in details [10–17]. In practice, the real-time SCMI nonoptical images are acquired and displayed on a computer screen where capacitance values are presented as pixels in a range of 256 gray levels. When a close contact is secured between the probe and the skin surface, the darker pixels correspond to high capacitance (moisturized) spots, and the clear ones to lower capacitance values. The SCMI-derived mean gray level (MGL) is representative of the average skin surface hydration [12].

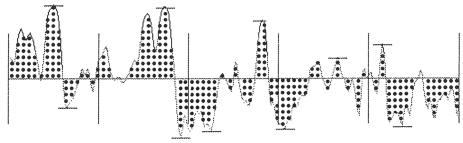


Figure 1: Profilometric parameters of skin microrelief. Ra is the dotted area, RN is the number of peaks above 0.1 mm, and Rz is the mean depth of the roughness profile (difference between the highest peak and deeper furrow in each of the 5 sectors).

The purpose of this study was to rate clinically and to assess objectively some variations in the skin surface landmarks on the dorsal hands of menopausal women over a 11-month period. The women received or not hormone replacement therapy (HRT). The heterogeneity in SC hydration and the skin microrelief were assessed by combining SCMI image analysis and the SCOP surface-shadowing.

2. Material and Methods

2.1. Design. The present observational study was approved by the Ethic Committee of the University Hospital of Liège, and the procedure was performed in accordance with the Declaration of Helsinki. The clinical and noninvasive instrumental procedures were conducted with the understanding and consent of all volunteers.

A total of 60 women with predominant outdoor occupational activities in open-air markets were enrolled in the study. For various reasons, not all subjects attended every assessment, and 13 of them did not completed the full program of the study. Among the 47 women who completed the study, 31 (66%) volunteers aged 53 ± 2 years received HRT. The other group of 16 volunteers aged 51 ± 3 years were out of HRT. The drop-out subjects were 5 women on HRT and 8 out of HRT. None of the women were using anytime hand creams and over-the-counter topical medications. There was no oral supplementation and a washout period was not necessary before starting the study. They were not usually wearing gloves. Four quarterly clinical and noninvasive instrumental assessments were performed starting in June-July. These evaluations corresponded to the summer, fall, winter, and spring periods, respectively. In each subject, the mid part of the back of the dominant hand was assessed clinically using both SCMI and SCOP. The participants of the HRT and non-HRT groups were blinded for the assessments at each seasonal collection.

On attendance for assessment, each subject first had to remain relaxed for 20–35 min in the Laboratory of Skin Bioengineering and Imaging under controlled temperature ($20 \pm 1^{\circ}$C) and humidity ($55 \pm 2\%$). In a first assessment step, a SkinChip (L'Oréal, Paris, France) probe was applied to the skin for 5 s at the most. The SCMI determination was obtained by the SkinChip device providing images of skin capacitance measurements every 50 μm.

In a second assessment step, a negative silicon replica (Silflo resin, Flexico Development Lim, Herts, UK) was collected as previously described [18, 19]. Each sample was illuminated by a floodlight (Highlight 3000 Olympus, Omnilabo, Brussels, Belgium) oriented at a 38° incidence angle. The lighting generated shadows, the width of which reflected the height of peaks and crests of the skin withering. A computerized image analyzer (Dermatec, Paris, France), working with gray-level discrimination, recorded surface topography parameters (Figure 1) Ra represented the mean roughness value, that is, the area above and below an average line through the center of the profile; Rz was the mean depth of roughness, that is, the average difference between the minimum and maximum heights in each of 5 adjacent sectors; Rn corresponded to the number of peaks or crests greater than 100 μm.

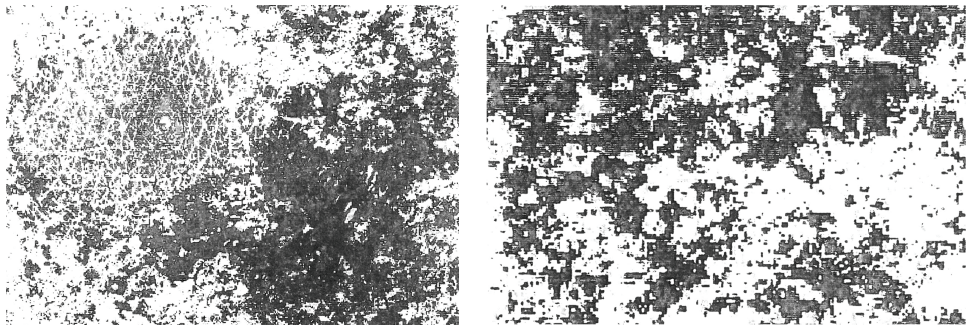
Following the biometeorological evaluations, a visual assessment was further performed. Xerotic and flaking skin was quite easily visualized after removing skin surface lipids. This was achieved following a swabbing method using cotton wool with propan-2-ol and allowing the excess alcohol on the skin surface to evaporate during 4 minutes before visual assessment [3].

The same trained assessor performed each assessment in order to guarantee continuity of scoring. The reproducibility of these records was periodically checked by comparing these scores with those given by a second expert assessor. Flaking xerotic skin was assessed using a predetermined scale (Table 1). This scale helped visual assessments of a series of parameters by examining three distinct visual aspects of flaking skin independently: (a) the grade of skin flakes, (b) their density, and (c) the area covered by the flakes. The clinical flaking score (CFS) was calculated by adding scores for each of the three aspects [3].

Table 1: Subjective scoring scale of flaking skin*.

Flake size
0: Dulled powdery appearance, no flakes visible
1: Very small but visible flakes
2: Intermediate sized flakes (at least two times size of I)
3: Large flakes with obvious curling edges
Flake density
0: No flakes, powdery only
1: Sparsely distributed flakes (size I to 3)
2: Nonuniform covering of flakes
3: Flakes in close proximity, with uniform covering of flaking area
Area of cover
0: No flaking
1: Less than one-third of swabbed area flaking
2: Once- to- two-thirds of swabbed area flaking
3: Over two-thirds of swabbed area flaking

*Flaking score = sum of scores of flake size, density, and area of cover. Possible scores: 0, 3, 4, 5, 6, 7, 8, 9.



(a) HRT menopausal woman (b) Menopausal woman out of HRT

Figure 2: Examples of heterogeneous patterns of skin capacitance mapping on dorsal hands in winter.

Data were expressed as means and standard deviations (SD). The minimum and maximum values were recorded as well as the medians. The Student's t-test and the Kruskal-Wallis test were used for comparing the two volunteer groups in the time-related observations. The relationships between the recorded parameters at each evaluation time were assessed using both the Pearson and the nonparametric Spearman correlation coefficients. The seasonal effect for each group of volunteers was assessed using the two-way analysis of variance (ANOVA-2). Multiple comparisons were performed according to the Scheffe test. The initial summer assessments in June-July served as references. Results were considered significant at the 5% critical level ($P < 0.05$). Calculations were performed using the SAS version 9.3 software (SAS Institute, Cary, NC, USA).

3. Results

Data about Ra, Rn, Rz, and SCMI-MGL are presented in Tables 2, 3, 4, and 5. The SCMI showed heterogeneous patterns of pixel darkness, particularly at the winter assessment in about 25–35% of women irrespective of their HRT status (Figure 2). For all instrumental assessments a periodic seasonal change during this study was clearly evidenced in most volunteers.

3.1. Seasonal Effect. The average CFS fluctuated over the seasons. In menopausal women out of HRT, it reached 3.1 ± 0.7 in summer, 3.9 ± 1.3 at fall, 7.4 ± 1.2 in winter, and 4.4 ± 2.3 in spring. In HRT recipients, the CFS averaged 3.3 ± 0.5 in summer, 3.5 ± 0.6 at fall, 6.3 ± 0.8 in winter, and 4.0 ± 1.6 in spring.

SCMI-MGL showed seasonal fluctuations in women receiving HRT ($P = 0.0014$) or not ($P = 0.0002$). Ra showed no seasonal variations in HRT women. By contrast, a prominent effect ($P < 0.0001$) was yielded on Ra in women out of HRT. Rn showed no seasonal effect in HRT women. By contrast, a major effect ($P < 0.001$) was observed in women out of HRT. Rz showed seasonal variations in women receiving HRT ($P = 0.047$) or not ($P < 0.0001$).

When considering both groups of women, SCMI-MGL showed a seasonal effect ($P < 0.0001$) without any group difference. The evaluations of each roughness parameter were different in each women group for Ra ($P < 0.001$), Rn ($P < 0.001$), and Rz ($P = 0.013$).

3.2. Intergroup Comparisons at Each Season. No significant differences were yielded between the CFS of the two groups of women anytime during the study. By contrast, some significant intergroup differences were present in the biometeorological assessments.

During summer, no intergroup differences were yielded for Rz and SCMI-MGL. By contrast, both Ra and Rn values were significantly higher ($P = 0.012$ and 0.0071, resp.) in the HRT recipients.

At fall, significant differences were present in each of the four parameters between the two groups of women. The SCMI-MGL was higher ($P = 0.0021$) in women out of HRT. By contrast, the roughness parameters had higher values in the HRT group (Ra, $P = 0.0024$; Rn, $P = 0.0082$; Rz, $P = 0.0002$).

In winter, no difference was observed between the two groups regarding Rz and SCMI-MGL. By contrast, both Ra ($P = 0.024$) and Rn ($P = 0.012$) were higher in the nonsupplemented women.

In spring, no significant differences were yielded for Ra and Rz between the two groups. Values of Rn ($P = 0.036$) and SCMI-MGL ($P = 0.012$) were higher in women out of HRT.

3.3. Correlations between Skin Capacitance and Roughness Parameters in both Groups at Each Season. During summer, HRT women showing high SCMI-MGL were significantly associated with low roughness (Ra, $P = 0.0392$; Rn, $P < 0.001$; Rz, $P < 0.0001$). In women out of HRT, a high SCM-IMGL was associated with low values of Ra ($P = 0.0013$) and Rz ($P < 0.0001$).

At fall, HRT women exhibiting high SCMI-MGL exhibited low values of Ra ($P = 0.0004$), Rn ($P < 0.0001$), and Rz ($P < 0.0001$). In women out of HRT, a positive correlation was found between Ra and Rn ($P = 0.029$) and between Rn and Rz ($P = 0.007$).

Table 2: Ra values.

Season	*N*	Mean	SD	Min	Median	Max
			HRT			
Summer	31	9.57	4.45	2.62	9.46	19.27
Fall	31	8.60	3.71	2.38	8.09	16.58
Winter	31	8.89	4.24	1.99	8.59	16.95
Spring	31	9.12	3.73	2.24	8.48	15.59
			Non-HRT			
Summer	16	6.35	2.88	1.98	6.06	12.13
Fall	16	5.37	2.12	1.73	5.37	8.38
Winter	16	12.18	5.14	8.10	10.14	28.42
Spring	16	10.82	2.64	6.33	10.05	16.27

Table 3: Rn values.

Season	*N*	Mean	SD	Min	Median	Max
			HRT			
Summer	31	3.71	4.02	0.00	2.00	13.00
Fall	31	5.74	5.28	0.00	4.00	17.00
Winter	31	5.77	4.67	0.00	6.00	14.00
Spring	31	5.26	4.65	0.00	3.00	13.00
			Non-HRT			
Summer	16	0.81	1.05	0.00	1.00	4.00
Fall	16	2.00	1.51	0.00	2.00	5.00
Winter	16	9.38	4.06	0.00	10.50	14.00
Spring	16	9.31	3.42	3.00	9.00	15.00

Table 4: Rz values.

Season	*N*	Mean	SD	Min	Median	Max
			HRT			
Summer	31	18.63	10.36	5.80	17.80	46.60
Fall	31	23.37	14.08	9.40	17.80	59.50
Winter	31	27.09	17.70	5.80	18.40	63.20
Spring	31	23.05	15.00	5.80	18.00	55.30
			Non-HRT			
Summer	16	13.23	3.94	7.80	13.00	21.40
Fall	16	11.36	3.46	6.80	10.60	17.00
Winter	16	28.40	14.28	4.02	30.25	58.40
Spring	16	24.39	11.73	8.40	21.75	47.60

Table 5: Non-optical capacitance imaging (mean gray level).

Season	*N*	Mean	SD	Min	Median	Max
			HRT			
Summer	31	122.61	36.96	64.00	129.00	186.00
Fall	31	109.29	34.95	57.00	115.00	176.00
Winter	31	92.35	37.40	29.00	101.00	153.00
Spring	31	104.19	36.75	50.00	96.00	163.00
			Non-HRT			
Summer	16	127.94	25.01	74.00	130.00	170.00
Fall	16	138.75	11.46	122.00	138.00	161.00
Winter	16	105.94	27.38	68.00	106.50	150.00
Spring	16	129.19	13.61	109.00	130.50	157.00

In winter, HRT women with high SCMI-MGL had low values of Ra ($P < 0.0001$), Rn ($P < 0.0001$), and Rz ($P < 0.0001$). The women out of HRT showing high SCMI-MGL had a low Rz value ($P = 0.0016$).

In spring, HRT women with high HCMI-MGL had low values of Rz ($r < 0.001$). No correlations were found between SCMI-MGL and any roughness parameter in women out of RHT.

4. Discussion

The normal SC binds water for ensuring a soft and smooth surface. It corresponds to a sturdy tissue of tightly packed coherent corneocytes. Its components have the capacity to bind water and to prevent water evaporation from the skin surface. The upshot of previous intense investigations was the perception that the dead, anucleated corneocytes remained indeed active metabolically. As corneocytes move to the skin surface, a remarkable series of structural and enzymatic changes take place [1, 2]. These events are tightly regulated and ensure homeostasis facing the hostile physical and chemical environment. Some abnormalities in such SC processing produce a xerotic and rough skin surface potentially leading to fine cracking and fissuring.

Many intrinsic factors influence the appearance and function of the skin surface. Similarly, many extrinsic environmental factors exert a physiological effect either directly or indirectly. For instance, xerotic conditions occur more commonly during winter season. The direction of the environmental temperature variations is opposite to the change in global flaking. Many studies attempted to attribute skin dryness and chapping to further environmental factors including relative humidity and absolute humidity. Atmospheric moisture and dew point are thus expected to exert some influences on the skin condition [3, 20, 21].

It is a common place for laypeople to complain from "dry skin" during cold seasons. Obviously, the geographic environment and meteorology contribute to the skin ailment. Few studies have been performed in the past for objectivating the skin surface impact of the successive seasons. The present study was undertaken on specific groups of early menopausal women receiving or not HRT. The HRT supplementation indeed exerts some effects on the skin [22, 23]. The objective methods were the quantifications of the SC hydration (skin capacitance) and skin withering/roughness/harshness using clinical ratings as well as determinant of skin capacitance and the Ra, Rn, and Rz parameters on skin replicas. All these procedures were noninvasive. In most seasons and in both women groups, the roughness parameters Ra, Rn, and Rz appeared correlated in the present study. A high capacitance level was commonly associated with a discrete roughness in HRT recipients at any season. Such correlations were less obvious in women out of HRT.

Clinical ratings from subjective assessment scales should never use any more than a five-point descriptor scale, because assessor proficiency steadily decreases as the number of assessment classes increases above this level. The composite scale presently used enabled a representative assessment of the skin condition [3]. The clinical rating globally yielded seasonal variations consistent with the volunteer perceptions of a xerotic aspect of the dorsal hands during successive seasons. Contrary to common expectations, the instrumental assessment were at variance, particularly in the distinction of skin reactivity according to the intake or avoidance of HRT.

The present study clearly distinguishes a different reactivity of the SC of menopausal women to environmental changes according to the intake or not of HRT. Seasonal variations appeared less impressive in HRT recipients. Surprisingly, during summertime, both Ra and Rn were more pronounced in the HRT women than in nonsupplemented women. No difference was disclosed with women out of HRT as far as the mean skin capacitance and Rz were concerned. At fall, all four biometeorological parameters were affected differently in both groups of women. In particular, skin capacitance was higher in women out of HRT. During wintertime, both Ra and Rn became higher in women out of HRT. During that period, no intergroup difference was evidenced for both mean capacitance and Rz. In spring, both skin capacitance and Rn were higher in women out of RHT while no intergroup group differences were yielded for Ra and Rz. In sum, mean skin capacitance showed little differences between both groups of menopausal women. By contrast, parameters of skin roughness showed larger intergroup differences, and variations were in opposite directions during colder and warmer seasons.

In short, in the group of menopausal women out of HRT, the seasonal effect combined lower capacitance and increased roughness as shown by Ra, Rn, and Rz during winter. In HRT recipients, a similar chronobiology was evidenced for both skin capacitance and Rz. Hence, HRT appeared to abate some manifestations of seasonal dry skin on the dorsal aspect of the hands. In this study, the effects of the external environment were specifically considered. This study demonstrates seasonal changes in the prevalence of a xerotic flaking skin condition, and the incidence appears to be governed by environmental temperature and atmospheric moisture content. However, the indoor environment possibly contributed to the overall skin condition. Indeed, alternate exposures to both normalized indoor and variable natural outdoor environments undoubtedly put the skin under stress [3]. They probably affect its general condition, particularly under extreme temperatures and humidity. The xerotic flaking skin condition possibly results in part from such cyclic exposures.

Conflict of Interests

The authors state no conflict of interests.

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