Original Research Article

A retrospective study of 137 dysplastic nevi: Are a personal history of melanoma and other histopathological factors associated with high-grade cytologic atypia?

Abstract

Aim: The association between the clinical and histopathological features of dysplastic nevi (DN), and the risk of melanoma is not clearly known. Thus, the aim of the present study is to determine if there is an association between the clinical and histopathological features of DN, and grade of cytologic atypia and personal history of melanoma (PHM).

Study Design: Retrospective clinicopathological study.

Place and duration of Study: Departments of Dermatology and Pathology, Hacettepe University School of Medicine, between 2000 and 2010.

Methodology: The study included 137 DN in 85 patients. Clinical parameters, including age, gender, PHM and/or family history of melanoma, dysplastic naevus syndrome and lesion diameter and location, were retrospectively evaluated. Histopathological parameters, including presence of architectural changes, host response features were also evaluated, and cytologic atypia was graded as mild, moderate, or severe. Lastly, 2 DN subgroups were formed, as mild atypia and high-grade (moderate-severe) atypia. Statistical analysis was performed to identify any associations between atypia grade, and other DN features and PHM.

Results: Mean age of the patients was 32.49 ± 13.02 years and the female-male ratio was 45/40. Most of the DN had moderate (49.6%) and mild atypia (39.4%), whereas severe atypia was observed in 10.9% of the lesions. DN with high-grade atypia were observed more frequently in the female patients (P = 0.042). Extremity localization, bridging and horizontal orientation of nests were more common in DN with high-grade atypia (P = 0.047, P = 0.006,

P = 0.046, respectively). Furthermore, DN with high-grade atypia were associated with PHM, independent of all other factors (P = 0.026).

Conclusions: High-grade cytologic atypia in DN was associated with female gender, extremity location, bridging and horizontal orientation of nests, and PHM.

Keywords: *Dysplastic nevus; cytologic atypia; melanoma.*

Introduction

It is well known that melanoma can develop from dysplastic nevi (DN). In addition, the presence of DN is considered to be an independent risk factor for the development of de novo melanoma; therefore, diagnosis and proper management of DN are important [1, 2]. Nonetheless, much about DN remains unknown. Moreover, the diagnostic criteria for DN remain a contentious issue [3, 4] and there is yet no standard system for grading cytological atypia and dysplasia [2]. In addition, although the presence of DN is a risk factor for melanoma, the features of DN associated with the risk of melanoma remain unclear. The present study aimed to determine if grade of cytologic atypia and/or personal history of melanoma (PHM) are associated with the clinical and histopathological features in patients with DN.

Materials and Methods

The study included 118 patients seen between 2000 and 2010 with histopathologically confirmed diagnoses of 170 DN, whose data were stored in our institution's computer database. Clinical data were collected from the patients' medical charts and/or from the patients directly. Data collected included age at presentation, gender, PHM and/or family history of melanoma, presence of dysplastic naevus syndrome (DNS) and lesion diameter and location—grouped as posterior trunk, anterior trunk, lower extremity, upper extremity, head/neck, and hand/foot. Histopathological data were obtained from formalin-fixed, paraffin-

embedded sections stained with H&E that were reexamined by a dermatologist and a pathologist, independently from the initial diagnosis. DN were defined histopathologically by the presence of architectural disorder, together with cytologic atypia and host response features, as previously described [5]. DN with inadequate clinical and histopathological data (biopsy other than excisional biopsy, nevi with only cytologic atypia or architectural disorder) were excluded from the study; as such, 137 DN in 85 patients were included in the study.

As histopathological parameters, lesion diameter and type of melanocytic nevus (junctional or compound) were noted. The presence of maturation and focal pagetoid spread were also evaluated, as were the presence of architectural changes, host response features, and cytologic atypia grade, as follows:

1. Architectural features: The presence of shoulder phenomenon (junctional component extending at $\geq$ 3 rete ridges beyond the dermal component), lentiginous melanocytic hyperplasia (proliferation of melanocytes in the basal layer, predominantly as single cells), and distribution of nest organization—bridging (fusion of nests at adjacent rete ridges) and horizontal orientation of nests (the long axis of melanocytes in nests extending parallel to the epidermis);

2. Host response features: The presence of eosinophilic fibrosis (subepidermal fibrosis encircling rete ridges)/lamellar fibrosis (fibrosis as layers of collagen fibers), increased vascularity, lymphohistiocytic infiltration, and pigment incontinence;

3. Cytologic atypia was graded as mild, moderate, or severe, based on the morphological characteristics of the melanocytes, as previously described by Weinstock et. al. [6] Size of the nuclei, variability in the shape and size of nuclei, and nucleolar prominence were evaluated. If the size of a melanocyte nucleus was equal to or less than that of a basal keratinocyte nucleus, the shape and size variability was minimal, and the nucleolus was not prominent it was considered mild atypia, if the size of a melanocyte nucleus was 1-1.5-fold greater than that of

a basal keratinocyte nucleus, shape and size variability was marked, and the nucleolus was not prominent, it was considered as moderate atypia, if the size of a melanocyte nucleus was equal to or ≥ 2-fold that of a basal keratinocyte nucleus, size and shape were variable, and the nucleolus was prominent it was considered as severe atypia. Moreover, mild, moderate, or severe atypia was the grade if the highest degree atypia was present in ≥ 5 melanocytes in a high-power field [6].

Maturation was defined as melanocyte nuclei that became smaller with progressive descent into the dermis and was only evaluated in compound DN. Migration of melanocytes into the upper layers of the epidermis was considered to be focal pagetoid spread. According to grade of cytologic atypia, all DN were graded as mild, moderate, or severe. Additionally, 2 subgroups were formed, as DN with mild atypia and high-grade (moderate-severe) atypia, and clinical and histopathological features were compared between these subgroups.

Statistical analysis

Statistical analysis was performed using SPSS v.15.0 for Windows (SPSS, Inc., Chicago, IL, USA). Continuous variables are presented as mean ± SD, and categorical variables as frequency and percentage. The chi square test was used to determine associations between categorical variables. For normally distributed variables between-group differences were determined via the independent samples t-test, whereas the Mann Whitney U test was used for variables that were not normally distributed. More than 2 groups were compared using the Kruskal-Wallis test. The level of statistical significance was set at $P < 0.05$. Because of the small number of DN, locations were divided into 3 groups, as extremity, trunk, and head/neck, for further analysis. Multivariate logistic regression was performed to identify the independent factors associated with PHM and high-grade atypia. Hacettepe UniversityEthics Committee approved the study protocol.

Results

In all, there were 137 DN in 85 patients. Mean age of the patients was 32.49 ± 13.02 years (range: 12-77 years). Among the patients, 45 (52.9%) were female and 40 (47.1%) were male. Of the 137 DN, 69 (50.4%) were in male patients. In total, 13 patients (15.3%) were positive for PHM and 6 patients (7.1%) had a positive family history of melanoma and 14 patients (16.5%) had DNS. Of 137 DN, 49 (35.8%) were observed in DNS patients.

Lesion localization in the female and male patients did not differ significantly (P = 0.765); the most common lesion localization was the posterior trunk, both in males and females. Median lesion size was 5 mm (range: 4-15 mm) and 76.6% of the lesions were compound DN, of which 91.4% exhibited the shoulder phenomenon. Among the DN, 91.2% had lentiginous melanocytic hyperplasia; bridging of nests was observed in 114 (83.2%) of the DN, versus horizontal orientation of nests in 47 (34.3%).

Lamellar fibrosis was observed in more of the DN than was eosinophilic fibrosis (90.5% vs. 37.2%). Most of the DN had moderate and mild cytologic atypia (n = 68 [49.6%] and n = 54 [39.4%], respectively), whereas severe atypia was observed in only 15 (10.9%) lesions. In 93.3% of the compound DN maturation was observed and focal pagetoid spread was noted in only 3 lesions (2.2%). In DN without maturation and/or focal pagetoid spread the diagnosis of melanoma was excluded based on the absence of other features of melanoma.

DN with severe atypia were more common in the female patients (73.3% in females vs. 26.7% in males, P = 0.043). PHM was significantly more common in the patients whose DN had moderate atypia (P = 0.023). There weren't any significant differences between the patients with DN that had mild, moderate, and severe atypia with respect to other clinical factors, including age, family history of melanoma, and mean lesion diameter. DN with severe atypia were significantly more common on extremities (P = 0.047). Severe atypia was significantly correlated with horizontal orientation of nests (P = 0.013), bridging of nests (P = 0.012), loss of maturation (P = 0.017), and focal pagetoid spread (P = 0.001). Comparison of

DN according to cytological atypia grades is given in Table. After grouping moderate and severe atypia together as high-grade atypia and comparing mild and high-grade atypia groups there weren't any significant differences in clinical parameters, age distribution, mean lesion diameter, or presence of a family history of melanoma; however, DN with high-grade atypia were observed significantly more frequently in the female patients (P = 0.042) and PHM was significantly more common in the patients whose DN had high-grade atypia (22.9% in the high-grade atypia group vs. 9.3% in the mild atypia group, P = 0.04). Most of the lesions in both groups were located on the trunk, although extremity localization was more common in the high-grade atypia group (24.1% vs. 16.7%). The incidence of bridging (P = 0.006) and horizontal orientation (P = 0.046) of nests were significantly higher in lesions with high-grade atypia; the other features did not differ significantly between the 2 atypia groups. When the clinical features of DN in patients with PHM were compared with those in patients without PHM, extremity localization was significantly more common in the patients with PHM than in those without PHM (37.5% vs. 17.7%, P = 0.042), although the most common location of lesions in both groups was the trunk (62.5% vs. 76.1%). In addition, moderate cytologic atypia (P = 0.023) and bridging of nests (P = 0.013) were significantly more common in the patients with PHM. There were no significant histopathological differences between sporadic DN and DN associated with DNS (P > 0.05).

PHM (P = 0.042), severe atypia (P = 0.047), and focal pagetoid spread (P = 0.042) were significantly more common in patients with lesions located on extremities. Multivariate logistic regression analysis showed that high-grade atypia were associated with PHM independent of all other factors (OR: 3.64; 95% CI: 1.17-11.3; P = 0.026). Furthermore, multivariate analysis showed that bridging and horizontal orientation of nests were more common in DN with high-grade atypia (OR: 3.07; 95% CI: 1.15-8.22; P = 0.025 and OR: 1.52; 95% CI: 0.6-3.6; P = 0.035, respectively).

148 **Table. Comparison of DN according to cytological atypia grades.**

	Mild atypia n = 54 (%)	Moderate atypia n = 68 (%)	Severe atypia n = 15 (%)	P
Mean ± SD age (years)	32.1 ± 12.9	32.6 ± 11.5	31.8 ± 10.9	0.953
Male/Female	33/21	32 /36	4/11	**0.043**
PHM	5 (9.3)	18 (26.5)	1 (6.7)	**0.023**
DNS	22 (40.7)	25 (36.8)	2 (13.3)	0.109
Family history of melanoma	3 (9.1)	3 (7.7)	0 (0)	0.347
Localization				
Trunk	42 (77.8)	52 (76.5)	7 (46.7)	
Extremities	9 (16.7)	12 (17.6)	8 (53.3)	**0.047**
Head/neck	3 (5.6)	4 (5.9)	0 (0)	
Type				
Compound	43 (79.6)	54 (79.4)	8 (53.3)	0.107
Junctional	11 (20.4)	14 (20.6)	7 (46.7)	
Architectural features				
Shoulder phenomenon*	40 (93*)	49 (90.7*)	7 (87.5*)	0.852
Lentiginous melanocytic hyperplasia	47 (87)	64 (94.1)	14 (93.3)	0.381
Bridging of nests	39 (72.2)	60 (88.2)	15 (100)	**0.012**
Horizontal orientation of nests	14 (25.9)	23 (33.8)	10 (66.7)	**0.013**
Host response features				
Eosinophilic fibrosis	23 (42.6)	22 (32.4)	6 (40)	0.495
Lamellar fibrosis	46 (85.2)	64 (94.1)	14 (93.3)	0.229
Increased vascularity	39 (72.2)	51 (75)	14 (93.3)	0.162
Pigment incontinence	50 (92.6)	62 (91.2)	14 (93.3)	0.939
Lymphohistiocytic infiltration	28 (51.9)	37 (54.4)	10 (66.7)	0.593
Maturation*	42 (97.7*)	51 (94.4*)	5 (62.5*)	**0.017**
Focal pagetoid spread	0 (0)	0 (0)	3 (20)	**0.001**

149 *Evaluated only for compound DN, and percentages represent the ratios within the compound

150 DN. Bold denotes significant difference ($P < 0.05$).

151 **Discussion**

152 DN remain a contentious issue, of which the first contentious issue concerns whether or not

153 cytologic atypia based on histopathological examination is a necessary diagnostic criterion [1,

7]. The1992 National Institutes of Health Consensus Conference defined the histopathological features of DN as architectural disorder with asymmetry, subepidermal fibroplasia, lentiginous melanocytic hyperplasia with nests of variable size, bridging of adjacent rete ridges, and the presence of the shoulder phenomenon. This consensus did not require cytologic atypia for the diagnosis of DN [1], however, some researchers, including Clark et al. [8] and Culpepper et al. [7], think that cytologic atypia must also be present for the diagnosis of DN because some degree of architectural disorder may be present in most nevi. Similarly, we think that considering both architectural disorder and cytologic atypia constitutes a more precise approach to the diagnosis of DN. As such, in the present study DN were diagnosed based on the combination of architectural and host response features, and cytologic atypia, as reported by Mckee and Calonje [5].

Although the association between DN and an increase in the risk of melanoma is well known, [9] the specific clinical and histopathological features of DN associated with the risk of melanoma remain unclear [9-11]. The association between grade of atypia and the risk of melanoma has been studied. Arumi-Uria et al. retrospectively reviewed 6275 nevi with architectural disorder and grouped them as mild (40%), moderate (26%), and severe (5%) cytologic atypia; PHM was observed in 5.7%, 8.1%, and 19.7% of the patients whose DN had mild, moderate, and severe atypia, respectively [12]. The researchers concluded that the risk of melanoma increases as the grade of atypia increases. Shors et al. biopsied the most clinically atypical nevi in a series of melanoma patients and controls, and histological dysplasia was assessed independently by 13 dermatopathologists as none, mild, moderate, and severe [13]. The relative risk of melanoma was greater in the patients with moderate and severe DN. In the present study high-grade (moderate-severe) cytologic atypia was more frequently observed in patients with PHM. Furthermore, multivariate analysis showed that high-grade atypia increased the risk of PHM 4-fold. Although more patients that have DN

with severe atypia can be expected to have PHM than those whose DN have moderate atypia, the present findings are not in agreement—most likely due to the small number of patients with DN that had severe atypia. As DN with severe atypia are less frequently diagnosed, we think it might be more practical to categorize atypia as mild and high-grade when evaluating the risk of melanoma. The present findings show that DN with high-grade atypia were associated with an increased risk of melanoma.

The correlation between architectural and host response features, and cytologic atypia is another contentious DN issue. The literature includes a limited number of studies on the correlation between the degree of atypia and other histopathological features of DN. Balkau et al. studied 334 melanocytic lesions and reported that architectural features, such as border irregularity, elongation of nests, variability in the number of melanocytes in the basal layer, and bridging of nests, were more commonly observed in lesions with cytologic atypia [14]. Barnhill et al. studied 153 atypical lesions in patients with PHM and observed that basal melanocytic hyperplasia, disarray of junctional nests, prominent vascularity, and large melanin granules were correlated, and that the presence of melanophages were inversely correlated with nuclear atypia, based on multivariate analysis [15]. Shea et al. reported that the degree of architectural disorder and the degree cytologic atypia were positively correlated, but they did not group DN according to grade of atypia [16]. Babacan and Lebe reported a similarly significant relationship between the degree of architectural disorder and cytologic atypia, and also reported that the presence of dermal fibroplasia (concentric or lamellar) correlates with the degree of architectural disorder and cytologic atypia [17]. In the present study only bridging of nests and horizontal orientation of nests were associated with high-grade (moderate-severe) atypia.

Gender- and site-specific histopathological features of DN are new concepts. Coras et al. observed that cytologic atypia was significantly more common in DN localized on the lower

legs in females [18]. Although the small number of DN located on lower legs in the present study precluded comparison of DN located on the lower legs and other localizations, it was observed that high-grade atypia was more common in the DN located on extremities. In addition, the incidence of DN with high-grade atypia was more common in the present study's female patients. The cause of the observed location- and gender-associated differences were not discerned, but we think they could be related to hormonal factors or external factors such as UV exposure which need to be clarified with further research.

The limitations of the present study include its retrospective design and the small number of patients. In conclusion, horizontal orientation and bridging of nests were histopathological features associated with high-grade cytologic atypia. DN with high-grade atypia were associated with extremity localization, female gender and PHM. As DN in the patients with PHM had high-grade atypia, we think that atypia grade might be used to identify and inform the management of patients with an increased risk of melanoma.

Ethical Approval

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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