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CLINICOPATHOLOGICAL STUDY OF MELANOMA WITH REFERENCE TO ITS POSSIBLE VIRAL ETIOLOGY

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Abstract

Background: The number of melanoma cases worldwide is increasing. In Egypt, skin cancer is uncommon malignancy. It represents 5 % of the malignant tumors, 8% of these were melanomas. The histogenesis of melanoma is still in dispute. Human Papilloma Viruses (HPV) are said to be responsible for the development of skin cancer and may play a role in the development of melanoma. This study is a trial to investigate melanoma clinically, histochemichally and immunohistochemically, to clarify its pathogenesis, and I to investigate the possible role of viruses in its etiology.

Methods: 40 cases of Melanoma were retrieved from Oral pathology & Maxillofacial departments, faculty of dentistry,. University of Alexandria and the Central Research Institute of Stomatology. Moscow. From 40 cases, 15 were oral and 25 were from head and neck region. All cases were AJCC stage III and IV. Special stains were used including Masson-Fontana and Machiavelli's, techniques. Immunohistochemical techniques were applied using Melanoma (gp 100) Ab-1 (clone HMB 45)(cat.#AP-9003)and Human Papilloma Virus (HPV)Ab-3(Clone-K1H8) (cat.#AP-9003).

Results: An interesting finding was the presence of clear cells derived from melanoblasts not forming melanin, with little mitotic change that can pose clinical challenges in early diagnosis. Calcified bodies either intra or extra cellular, organized or non organized were detected, could be considered as one of the criteria of malignancy.

Conclusion: Viral inclusion bodies were observed and were confirmed using HPV antibody in five of the cases which might indicate a possible viral etiology.

Keyword- Melanoma, special stains, Human Papilloma virus

Background

Malignant melanoma is a malignant tumor arising from melanocytes. Its incidence and overall mortality rate have been rising in recent decades [1].

In Egypt, skin cancer is uncommon malignancy as compared to western societies. It represents 5 % of the malignant tumors, 8% of these were melanoma [2].

The histogenesis of melanoma is still in dispute. Approximately, 15% of all malignant melanomas occur in the head and neck region and about 5% arise in the oral mucosa. Ultraviolet (UV) radiation appears to be the primary causal agent in its development [3]. From a cellular and molecular stand point, UV radiation, have been shown to be associated with the development of some mutation in vitro. Only a few of these mutations are associated with cell cycle regulation, especially P53 regulatory protein in melanoma. These observations suggest that other agents may be involved directly or in directly in the development and progression of melanoma [4]. Other agents, especially viruses play a role in the development and progression of some skin cancers. Human Papilloma Viruses (HPV) are said to be responsible for the development of skin cancer and may play a role in the development of melanoma [4,5]. This study is a trial to investigate melanoma clinically, histochemichally and immunohistochemically, to clarify its pathogenesis and finally, to investigate the possible role of viruses in its etiology.

Materials

40 cases of melanoma were retrieved from: Oral pathology department, Maxillofacial department faculty of dentistry, university of Alexandria and The Central Research Institute of Stomatology. Moscow from the 40 cases of melanoma, 15 were from the oral mucosa and 25 were from head and neck region. All cases were (AJCC stage III and IV) evaluated using standard modalities, including physical examination, computerized tomography and magnetic resonances imaging. A portion of the tumor was used for research after informed consent was obtained.(According to WMA declaration of Helsinkiof ethical principles for medical research involving human subjects).

Histological and Histochemical Study

Each biopsy specimen was treated similarly. Routine hematoxylin and eosin stained sections were studied to confirm the diagnosis and to analyze the following features: ulceration, predominant cell type, rate of mitosis, presence of tumor giant cells and the presence of lymphocytes and plasma cells. The special stains using histochemical technique were:

- Gomori's reticulin impregnation technique
- Grocott hexamine-silver variant
- Machiavelli's technique for viral inclusion bodies
- Masson-Fontana for melanin

Immunohitochemical Study

After deparaffinization, serial sections (5 µ thick) were used. Sections were boiled in 10 mµ citrate buffer and subjected to the following:

- Immunohistochemical stains (according to the manufacturers recommendations).
- Melanoma (gp 100) Ab-1 (clone HMB-45) manufactured by Neomarkers for lab vision cooperation (Cat. # AP. 9003). Femront USA, to determine the presence of melanoma cells.
- Human papilloma virus (HPV) Ab-3 (clone K1 H8) manufactured by Neomarkers for lab vision cooperation (Cat. # AP. 9003). Femront USA, for detection of HPV type16, 18, 31, 33.

Statistical Analysis

Patients characteristics, including age, sex, AJCC stage and HPV status of tumor tissues were tested using chi-square test.

Results

Clinical Results

15 biopsy specimens were obtained from oral mucosa (37.5%) and 25 from head and neck region (62.5%).

The commonest intra oral melanoma was in the palate (7 cases) followed by alveolar mucosa (4 cases), buccal mucosa (3cases) and only one case in soft palate [Fig-1].

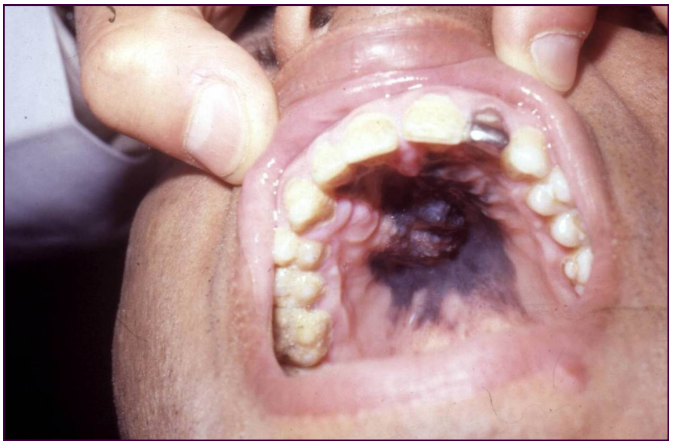

Fig. 1- Clinical picture of melanoma in the palatal mucosa

31 biopsy specimens were obtained from patients with AJCC stage III melanoma and 9 from patients with stage IV [Fig- 2].

The male to female ratio was 2.63: 1. Median age was 58.5 years (range 34.7-81.6).

Histological Results

Many calcified degenerated malignant cells of variable sizes and different degree of mineralization were found intra or extracellular, as well as calcific patches of necrotic foci in the form of globules or calcospherites [Fig-3].

Increase in normal as well as abnormal mitosis was seen. The mean mitotic figures per field were five [Fig-4].

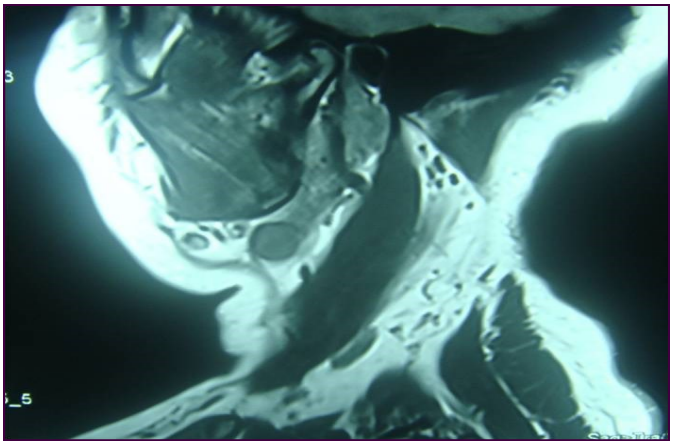

Fig. 2- MRI sagital view showing metastatic cervical lymph node

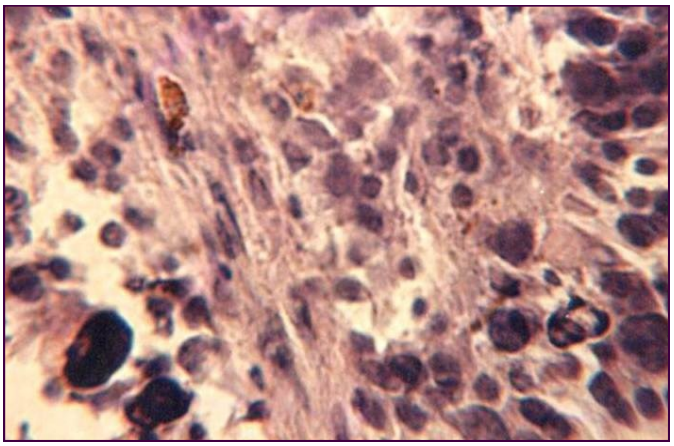

Fig. 3- Intracellular and extracellular calcifications in malignant cells (H&E x400)

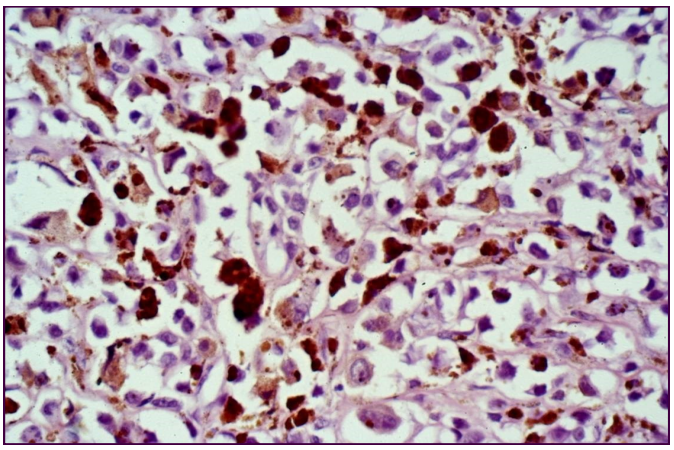

Fig. 4- Photomicrograph of melanoma showing polygonal and fusiform cells. Notice the abnormal mitotic figures (H & E x400)

Tumor giant cells were noticed in one of our cases with abnormal mitosis [Fig-5].

Primary malignant melanoma of the oral cavity showed marked tendency to metastasize to regional lymph node this was quite obvious in five of our cases [Fig-6].

An uncommon histological finding was the presence of clear cells only in the metastasis (one case). These clear balloon cells are

large with abundant vacuolated cytoplasm. Limited mitosis and scanty melanin were seen [Fig-7].

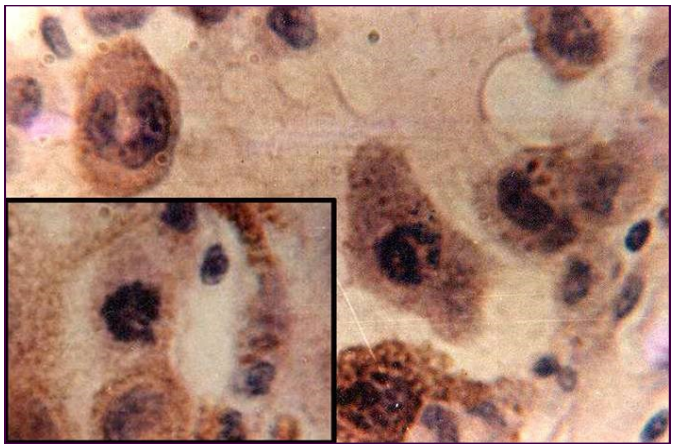

Fig. 5- Malignant tumor giant cells revealing abnormal mitosis and melanin pigments (H&E x400)

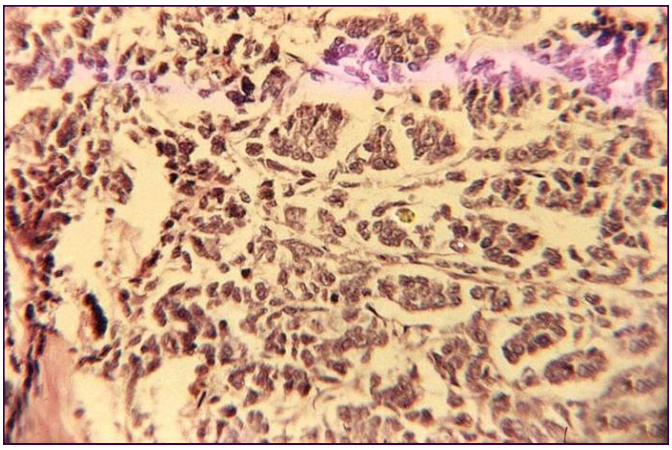

Fig. 6- Vascular invasion of melanoma cells bordered by the endothelial cells of the vessels (H&E x400)

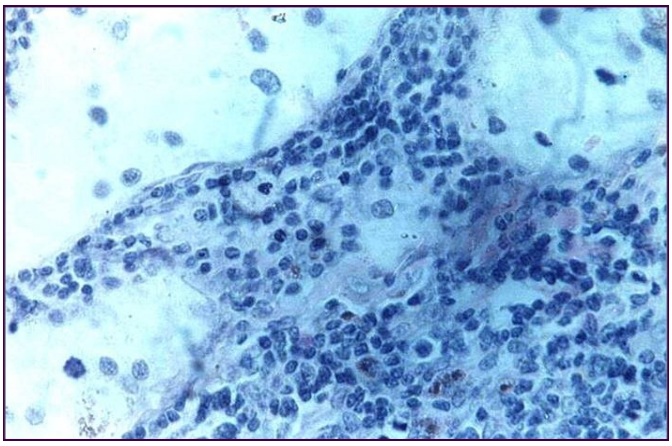

Fig. 7- Balloon cell melanobastoma with scanty melanin pigmentation and negative reaction for viral inclusion bodies. (Macchiavello's technique x400)

Histochemical Results

Gomori's Reticulin Impregnation Technique

This technique revealed the carcinoid arrangement of malignant melanoma in the form of nests and follicular pattern [Fig-8].

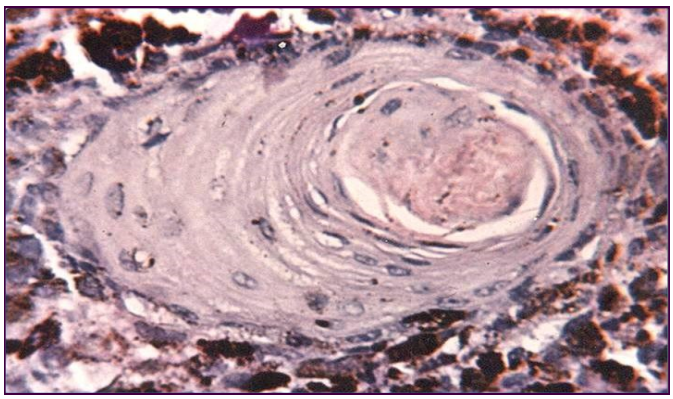

Fig. 8- Carcinoid arrangement of malignant melanoma (Gomori's reticulin stainx400).

Grocott Hexamine-Silver Variant

This is a specific stain for all the forms of infection. The organism will appear black and the back ground green. Unfortunately melanin pigments mask any.

Microorganism Detection

Masson-Fontana Technique

This was used to confirm the diagnosis of amelanotic melanoma [Fig-9].

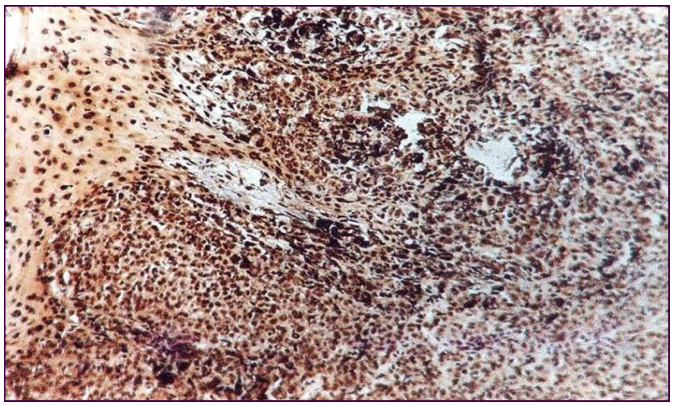

Fig. 9- Black color diagnosing the presence of melanin pigments within a brown background (Masson-Fontana technique x 200)

Machiavelli's Technique for Viral Inclusion Bodies

Five cases (12.5%) revealed positive magenta color in blue background indicating the presence of viral inclusion bodies [Fig-10].

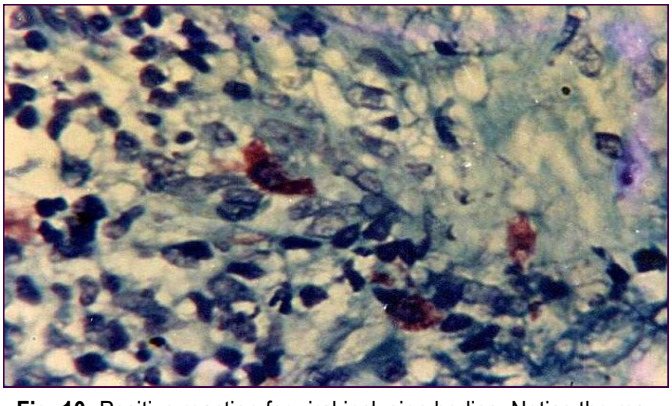

Fig. 10- Positive reaction for viral inclusion bodies. Notice the magenta color of infected cells (Machiavelli's technique x400)

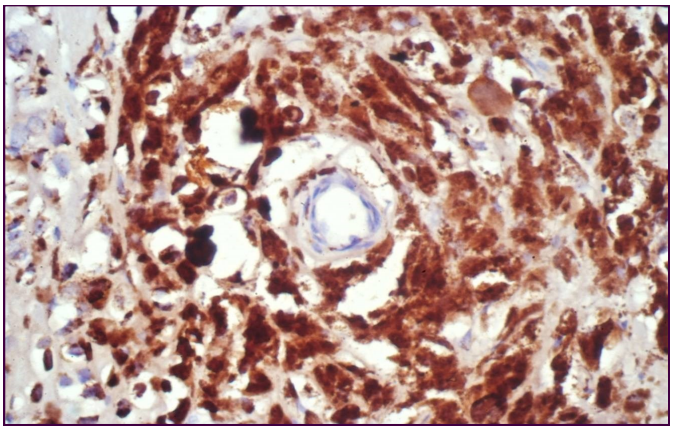

Fig. 11- Photomicrograph showing positive HMB-45 antibody. Cellular localization was cytoplasmic. (x400)

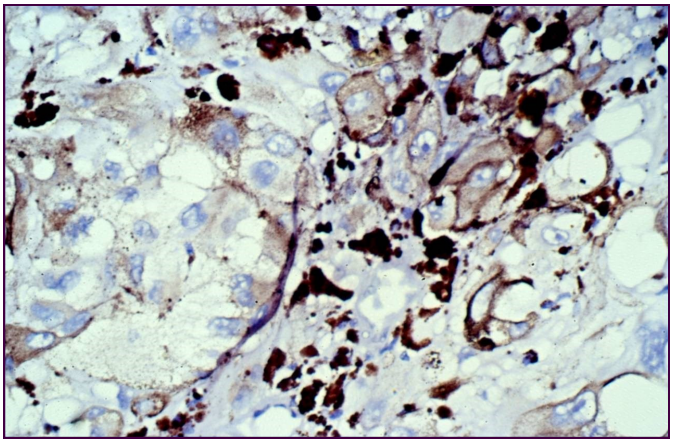

Fig. 12- Photomicrograph showing positive reaction for (HPV) antibody with cellular localization in the nucleus (400)

Immunohistochemical Results

All biopsy specimens were positive for HMB-45. Cellular localization was cytoplasmic [Fig-11].

The same cases that were positive for Machiavelli's technique for viral inclusion bodies were positive for (HPV) antibody with cellular localization in the nucleus [Fig-12].

Discussion

The incidence of cutaneous malignant melanoma has been increasing at a steady rate in fair-skinned populations around the world for decades. On the other hand mucosal melanomas of the head and neck are a rare entity, occurring much less frequently than their cutaneous counterparts [6]. In this study, among the studied 40 cases of melanoma, 15 biopsy specimens were obtained from oral mucosa (37.5%) and 25 from head and neck region (62.5%). The commonest intra oral melanoma was found in the palate, This goes with the results observed by Femiano F. and others who have found that the most frequent site of occurrence of oral melanoma is the hard palate followed by the maxillary gingival [7,8-9].Followed by the other oral sites include the mandibular gingiva, buccal mucosa and floor of the mouth [10].

Histologically, calcified degenerated malignant cells were found intra or extracellular, which could be considered as one of the criteria of malignancy [11].

Additionally, there was an Increase in normal as well as abnormal mitosis, with five mean mitotic figures per field [3].

One of our findings was the presence of Tumor giant cells with abnormal mitosis.

Philip, et al [12], had showed the same result but only in metastatic melanoma and not in the primary lesion.

In this study, It was noticed that, unlike their cutaneous counterparts, head and neck mucosal malignant melanomas behave much more aggressively as it was recorded that primary malignant melanoma of the oral cavity showed marked tendency to metastasize to regional lymph node. This was quite obvious in five of our cases [13,14].

An Interesting histological finding was the presence large clear balloon cells with abundant vacuolated cytoplasm, limited mitosis and scanty melanin. The differential diagnosis of this metastatic balloon cell malignant melanoma is broad and clinicopathologic correlation may play a critical role in achieving the correct diagnosis. Accordingly, immunohistochemical analysis is considered to be one of the most accurate tools for its diagnosis. This was in agreement with Lee et al [15], who had reported that Balloon cell malignant melanoma is the rarest histological type of primary cutaneous melanoma. These balloon cells are usually sparse or absent in the primary melanoma; He added that, when the balloon cell melanoma metastasizes, the metastases are often composed entirely of balloon cells with no residual spindle-shaped or epithelioid component.

In this research, varies histochemical and immunohistochemical stains were used for further studying as well as understanding the nature of melanoma.

Among these histochemical stains was Gomori's reticulin impregnation technique. This technique revealed the carcinoid arrangement of malignant melanoma in the form of nests and follicular pattern that mimic the squamous cell carcinoma [16]

Machiavelli's technique for viral inclusion bodies was used as well. Five cases revealed positive magenta color in blue background indicating the presence of viral inclusion bodies and were confirmed immunohistochmically being HPV, denoting its possible role in the etiology of melanoma. In a study done by Didier Dréau [4], it was found the presence of HPV in 58% of the biopsy specimens obtained from patients with stage III and IV melanoma and it was suggested that HPV may serve as a cofactor in the development of melanoma and may modulate a more aggressive phenotype in HPV -containing melanoma cells.

Although the number of biopsy specimens tested is small, the results still indicate a correlation between melanoma and the presence of HPV. Never the less, further studies on large number of patients with follow up are recommended.

Different approaches may be recommended as well to determine the presence of viral oncoproteins in biopsy specimens, including, polymerase chain reaction (PCR) and in situ hybridization.

Conclusion

1. The most common site for melanoma in the oral mucosa was the hard palate
2. The primary malignant melanomas of the oral cavity showed a tendency to metastasize to regional lymph nodes
3. Calcified bodies either intra or extra cellular, organized or non organized were detected, which could be considered as one of the criteria of malignancy.

4. Increase in normal, as well as abnormal mitosis was found. The mean mitotic figures per field were five.
5. Tumor giant cells were seen with abnormal mitosis
6. An interesting finding was the presence of clear cells derived from melanoblasts not forming melanin, with few mitotic changes that can pose clinical challenge in early diagnosis.
7. Balloon cells were only present in the metastatic foci. Their presence can be attributed to disturbance in melanin synthesis.
8. Carcinoid arrangement of malignant melanoma in the form of nests and follicular pattern were seen.
9. Viral inclusion bodies were proved in five cases and were confirmed being HPV, denoting its possible role in the etiology of melanoma

Abbreviations Used

HPV: Human Papilloma Virus

AJCC: American Joint Committee on Cancer

UV: Ultra Violet,

µ: Micron,

mµ: mill micron,

PCR: Polymerase Chain Reaction,

MRI: Magnetic Resonance Imaging,

H&E: Hematoxylin and Eosin

Competing Interests: There are no competing interests

Authors Contribution

Amani N.A. participated in the study design, collection of the background references, interpreting and displaying the results of the study, writing the discussion of the results, alignment of the references.

Abany M.H. participated in the study design, collection of the background references, Photomicrography of the histochemical and immunohistochemical results, interpreting and displaying the results of the study, writing the discussion of the results, alignment of the references.

Fata M.M. participated in selecting clinically the study cases follow up of these cases, interpreting and displaying the results of the study, writing the discussion of the results.

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