Immunohistochemical Detection of BRAFV600E in Cutaneous Melanocytic Neoplasms in a Cohort of Egyptian Population

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Abstract

Objective: Melanocytic neoplasia shows different pathways that have been implicated in tumorigenesis. One of the key genes involved in the BRAFV600E gene, can be detected through DNA-based or protein-based tests including immunohistochemistry (IHC). To check its reproducibility, this study was conducted to evaluate BRAFV600E IHC by different observers in a range of melanocytic neoplasms. Methods: The present study was performed to evaluate immunohistochemical staining (IHC) using the RM-08 to detect BRAF V600E in 50 formalin-fixed paraffin-embedded (FFPE) blocks of different melanocytic neoplasms after bleaching using Tris-HCL buffer. IHC was considered positive when more than 90% of tumour cells showed cytoplasmic staining. IHC assessment by a team of two pathologists and a third pathologist to check the interobserver variability. IHC scores were compared among nevi and melanoma. Results: IHC evaluation revealed good agreement by different observers (k-coefficient=0.691, p=0.00). BRAFV600E showed a statistically significant difference between nevi and melanoma. Conclusions: These findings suggest that BRAFV600E IHC assessment shows good reproducibility among pathologists, however, more strict criteria should be adopted in interpretation, especially in bleached samples

Keywords: BRAFV600E- melanoma- nevi- bleaching- egyptian
cancers have been identified, the most common of which is the BRAFV600E mutation, in which hydrophilic glutamic acid (E) substitutes hydrophobic valine (V) at codon 600. Less commonly encountered mutations are grouped as BRAF non-V600E mutations \[5\].

Mutational status is identified via molecular testing using different techniques, including the FDA-approved Cobas test, sequencing, and real-time PCR. Immunohistochemistry using a monoclonal antibody VE1 clone has been proposed as a surrogate for RT–PCR molecular testing \[5-7\]. However, interpretation of immunohistochemical staining is evaluated by multiple methods with interobserver variability.

In this study, BRAF V600E was assessed using IHC in 50 formalin-fixed paraffin-embedded (FFPE) sections of different melanocytic neoplasms by three observers to identify BRAF V600E status and limit the interobserver variability. The results of IHC testing were compared between melanoma and nevi to evaluate the diagnostic value of BRAF V600E.

**Materials and Methods**

**Patients**

The current work included 50 retrospective excisional or incisional biopsies of 29 melanoma cases retrieved from the Pathology Laboratory archives, Faculty of Medicine, Alexandria University starting from January 2017 to January 2020. Ethical approval was granted by the Faculty of Medicine Ethics Committee (IRB no. 00007555, FWA NO. 00015712).

**Immunohistochemical staining for BRAFV600E**

Four-micrometre-thick sections were cut from paraffin blocks and placed on coated slides. Melanin bleaching for 29 moderately and heavily pigmented cases using 0.5% diluted hydrogen peroxide (H\(_2\)O\(_2\)) in Tris-HCl and PBS was performed as described by Chung et al \[8\]. Antigen retrieval was performed using sodium citrate buffer in a microwave oven for 10 minutes. IHC staining was performed using Clone: RM-08, ThermoFisher Scientific, USA, dilution 1:300 in PBS according to the manufacturer’s protocol using a HrP kit (ThermoFisher Scientific) and diaminobenzidine (DAB) as a chromogen. positive and negative controls in the form of prostatic tissue and sections without primary antibody incubation, respectively.

**Statistical Analysis**

Statistical analysis was performed using SPSS version 20 (IBM) to evaluate interobserver variability and check the difference in BRAFV600E expression between nevi and melanoma.

**Results**

**Immunohistochemistry for BRAF in Melanoma**

Out of 29 melanoma cases, melanin bleaching before immunohistochemical staining was performed in moderately and heavily pigmented cases. In 16 cases with prior bleaching, BRAF immunohistochemical staining was labelled as positive in 9 cases. In 13 cases, with absent or minimal pigmentation, eight cases revealed positive staining in the form of cytoplasmic staining in tumour cells with a total of 17 melanoma cases positive for BRAF immunostaining.

The staining was almost homogeneous throughout tumour cells. Heterogeneous staining between different tumour regions was observed in the cases with prior bleaching, areas of necrosis, fixation artefacts or sometimes different tumour cell morphology.

Ten cases were considered negative due to the complete absence of staining or patchy staining of scattered tumour cells and interspersed macrophages. Only two cases were considered ambiguous, one case in which prior bleaching was done revealed intense nuclear and cytoplasmic staining and staining of wide necrotic areas. Nuclear staining was observed in the overlying epidermis and cytoplasmic staining in endogenous blood vessels (positive internal control).

Out of 21 nevi cases, only two cases showed positive cytoplasmic staining in tumour cells, one case was labelled histologically as pigmented epithelioid melanocytoma and revealed cytoplasmic staining in more than 80% of tumour cells after melanin pigment bleaching. The second case was dermal nevus with verrucous overlying epidermis that showed minimal pigmentation (score +1), this case showed moderately intense cytoplasmic staining. Nineteen cases showed absent staining or patchy weak staining in less than 5% of nevus cells. Different IHC staining patterns are shown in Figure 1.

**Immunohistochemistry assessment by an independent observer**

Using the same methodology, the third observer assessed the immunohistochemical staining in melanoma

![Figure 1. BRAFV600E Immunohistochemical Staining in Melanoma. A, Negative staining in tumour cells with positive internal control (black arrow); B, Ambiguous staining in tumour cells; C, Heterogeneous staining in tumour cells; D, Diffuse homogenous staining in tumour cells. (immunoperoxidase X200)](image-url)
Interpretation is crucial to avoid interobserver variability [6, 13]. Since 2011, multiple methods have been used to interpret the staining. The most commonly used was proposed by Capper et al [7, 13] and was used in the current work. Although, the method showed good reproducibility. Interpretation of heavily pigmented melanomas remained problematic, and a more strict method was proposed by Fisher et al [6]. Recently, a meta-analysis showed that intratumoral heterogeneity in BRAFV600E IHC might explain its lower sensitivity in BRAFV600E mutation detection [15]. Yancovitz et al [16] reported that intratumoral heterogeneity in BRAF expression caused a marked discrepancy between BRAFV600E molecular testing methods.

Nevertheless, the use of PCR-based tests was reported to be a more rapid, sensitive, specific and cost-effective method for detecting BRAF mutations [17-20].

In the present study, BRAF IHC expression showed a statistically significant difference between nevi and melanoma. BRAF V600E expression was found to be associated with tumour progression and a predictive marker of BRAF inhibitors [21].

In conclusions, the results obtained in this study indicate that the IHC method can be used as a screening tool for BRAFV600E; however, cannot be used as a surrogate marker for based tests including CAST-PCR and sequencing. There is no consensus on BRAF IHC staining interpretation criteria among different study groups, which in turn questions the methodology that should be adopted for staining interpretation.

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**Approval**

The research is approved by the faculty of medicine,
at Alexandria university.

**Conflict of Interest**
None declared.

**Ethical Declaration**
Clinical data were collected and evaluated according to the approval of the Faculty of Medicine Ethics Committee, Alexandria University (IRB no. 00007555, FWA NO. 00015712). The study was conducted by the international standards of good clinical practice and with the 1964 Helsinki declaration and its later amendments.

**Authors Contribution**
Nada M. Yakout, Dina M. Abdallah, Doaa Abdelmonisf and Omayma El-Sakka participated in the study design and coordination. Nada M Yakout, Omayma El-Sakka and Dina M. Abdallah performed the immunohistochemical analysis. Doaa Abdelmonisf performed and interpreted CAST-PRC for data validation. Hassan Mahmoud Kholosy collected and provided all clinical data. Nada M Yakout and Omayma El-Sakka wrote the main manuscript text. Nada M. Yakout and Dina M. Abdallah Omayma El-Sakka prepared Figure 1. The authors have read and approved the final manuscript.

**Data Availability**
Raw data sharing does not apply to Immunohistochemistry as no datasets were generated.

**References**


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