Using risk factors for detection and prognostication of uveal melanoma

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The early detection of malignancy, particularly uveal melanoma, is crucial in protecting visual acuity, salvaging the eye, and preventing metastasis. Risk factors for early detection of uveal melanoma have been clearly delineated in the literature and allow identification of melanoma when it is tiny and simulates a nevus. These factors include thickness >2 mm, presence of subretinal fluid (SRF), symptoms, the orange pigment, margin near optic disc, acoustic hollowness, surrounding halo, and absence of drusen. The importance of early detection is realized when one considers melanoma thickness, as each millimeter increase in melanoma thickness imparts 5% increased risk for metastatic disease. Newer imaging modalities like enhanced depth imaging optical coherence tomography and fundus autoflouroscence facilitate in detection of SRF and orange pigment. Additional molecular biomarkers and cytological features have been identified which can predict the clinical behavior of a small melanocytic lesion. Features that suggest a poor prognosis include higher blood levels of tyrosinase m-RNA, vascular endothelial growth factor, insulin-like growth factor; monosomy 3 and gains in chromosome 8. Management of uveal melanoma includes enucleation (for large), local eye wall resection, brachytherapy, charged particle irradiation, and thermotherapy (for small to medium tumors). Although the role of a good clinical evaluation cannot be underestimated, it is advisable to evaluate the various radiological, molecular, and cytological features, to enhance the accuracy of early diagnosis and improved prognosis.

Key words: Autoflouroscence, enhanced depth imaging, eye, gene expression profiling, malignancy, metastasis, monosomy 3, nevus, optical coherence tomography, tumor, uveal melanoma

Uveal melanoma is the most common primary intraocular malignancy in adults. It also happens to be one of the few intraocular diseases which can prove fatal.[1] Like cutaneous melanoma, early detection of malignancy is crucial in preventing metastasis and saving the patients' life with appropriate therapy. A uveal melanoma can arise either *de novo* or from a nevus or congenital ocular melanocytosis.[2] Choroidal nevus has a variety of features depending on patient age [Fig. 1a-c]. Older patients are more likely to have multiple nevi (10%), slightly thicker lesion (mean = 1.6 mm) and greater number of drusen overlying the lesion (58%) as compared to young patient (2%, 1.2 mm and 11% respectively). Choroidal nevi have also been shown to have chronic features such as retinal pigment epithelial hyperplasia (7%) and retinal pigment epithelial atrophy (10%); however, they can also show features that overlap melanoma such as associated subretinal fluid (SRF) (9%) and overlying orange pigment (6%).[3]

Although the lesion is ophthalmoscopically visualized, because of these features, it can be considerably difficult to differentiate a nevus from a small choroidal melanoma. The management decision at this juncture is important due to the prognostic implications and psychological impact of the disease. Many studies have been undertaken to address this issue, and many authors have listed various risk factors [Tables 1-3], for proper identification and subsequent management of small choroidal melanoma. With the advent of molecular biology and cytogenetics, several biological and cytological markers have been identified which can further predict the prognosis and help the clinician with decision making.
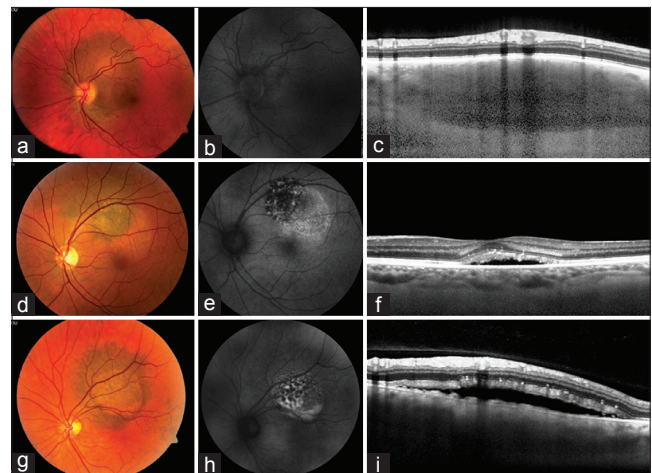

Figure 1: Clinical and imaging features of choroidal melanocytic lesions. Case 1: Circumpapillary pigmented choroidal lesion lacking orange pigment (a). Fundus autoflouroscense (b) is indistinct with no lipofuscin. Enhanced depth imaging-optical coherence tomography (c) normal photoreceptors and absence of SRF, consistent with choroidal nevus. Case 2: Pigmented choroidal lesion with overlying orange pigment, diffuse hyperautofluorescence (e) and the subfoveal fluid with shaggy photoreceptors (f) suggestive of choroidal melanoma. Case 3: Pigmented choroidal lesion with orange pigment (g), patchy hyperautofluorescence with sedimentation (h), and overlying SRF and shaggy photoreceptors (i), suggestive of choroidal melanoma

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Table 1: Host and environmental risk factors for melanoma

Host factors	Environmental factors
Caucasian	Arc welders
Oculodermal melanocytosis	Airline workers
Light irides	Sunlight

Table 2: Risk factors for early detection of melanoma

Clinical	Newer imaging techniques
T-Thickness >2 mm	Autoflourescence
F-Subretinal fluid	Hyperautofluorescence
S-Symptoms	
O-Presence of orange pigment	EDI-OCT
M-Margin within 3 mm of the disc	Increased tumor thickness
U-Ultrasound hollowness	Subretinal fluid
H-Halo absence	Subretinal lipofuscin
D-Drusen absence	Retinal irregularities
	Shaggy photoreceptors

EDI-OCT: Enhanced depth imaging optical coherence tomography

Table 3: Risk factors for metastasis from melanoma

Clinical
  Increasing age
  Tumor size-large
  Tumor growth
  Greatest basal dimension
  Oculo (dermal) melanocytosis
  Cilliary body tumor
  Brown/pigmented tumor
  Presence of subretinal fluid or intraocular hemorrhage
  Extraocular extension
Histopathological
  Cell type-epithelioid type
  Location-anteriorly placed
  Growth pattern-diffuse
  Mitotic figures
  Pigmentation
  Necrosis and inflammatory components prominence of vascularity
Molecular
  Tyrosinase m-RNA
  Vascular endothelial growth factor
  Hepatocyte growth factor
  Insulin-like growth factor-1
Cytological
  Chromosomal alterations
    Monosomy 3
    Gains in chromosome 8
  Gene alterations
    Mutations in *GNAQ*
    Mutations in *GNA11*

RNA: Ribonucleic acid

Host and environmental risk factors for melanoma

Host factors

It is important to look at the patient as a whole because ocular melanoma can be a part of a rather generalized disease. The presence of related ocular or cutaneous melanocytic lesions can be associated with a higher incidence of choroidal melanoma. The cutaneous conditions most frequently associated with uveal melanoma include primarily oculodermal melanocytosis (nevus of Ota) and rarely familial atypical mole and cutaneous melanoma.[4] Fair complexion and light irides are generally considered risk factors for uveal melanoma.[5] Weis *et al.* after their comprehensive meta-analysis, calculated the odds ratio of 1.75 (1.31-2.34) and 1.80 (1.31-2.47) for light irides and fair complexion, respectively, for the risk of developing uveal melanoma which was statistically significant.[6]

Environmental factors

Several environmental factors like sun exposure, and occupation like arc-welders are believed to be associated with an increased risk of ocular melanoma.[4,7]

Risk factors for early detection of melanoma

Early detection of a small choroidal melanoma can be a challenge and requires a detailed evaluation of the lesion, keeping in mind the subtle clinical features of the lesion suggestive of melanoma.

Clinical

Documented tumor growth has been widely accepted as a risk factor for malignant potential of the lesion.[8,9] Small pigmented lesions that grow have been shown to be malignant melanoma histopathologically.[10] But the growth is not always an indicator of malignancy.[8,11] Choroidal nevi can also show growth, but the amount and rate of growth are generally very little and over a long period of time. On the other hand, some melanomas remain clinically stable, and there have been cases of histopathologically confirmed melanoma that were stable for many years.[12]

In 1994, Collaborative Ocular Melanoma Study (COMS) group[13] reported that risk factors for growth and possible malignant transformation were:
- Greater initial tumor thickness and diameter
- Presence of orange pigment
- Absence of drusen, and
- Absence of areas of retinal pigment epithelial changes adjacent to the tumor.

In 1995, Shields *et al.*[14] statistically derived five risk factors for malignant behavior of small melanocytic choroidal lesions. These features were all based on routine funduscopic examination, making their use practical and significant. They proposed the use of mnemonic TFSOM (To Find Small Ocular Melanoma) to assist the clinician in early detection of small choroidal melanoma at risk for growth and metastasis [Fig. 1d-i]. TFSOM stands for:
- T-Thickness greater than 2 mm
- F-Subretinal fluid
- S-Symptoms
- O-Orange pigment present
- M-Margin within 3 mm of the disc.

In their study of 1287 patients,[15] they reported that if none of the above risk factors were present, growth was detected in only 4%. Tumor growth was documented in 30% and 41% of patients with one risk factor, 35% to 58% of patients with 2 risk factors, 36% to 63% of patients with 3 risk factors, and 39% to 62% of patients with 4 risk factors, and 56% of patients with all risk factors. The relative risk (RR) for growth was 1.6-2.3 for 1 factor, 2.8-5.0 for 2 factors, 5.5-9.6 for 3 factors, 11.6-17.3 for 4 factors, and 27.1 for all 5 risk factors combined. The greatest RR for growth occurred when all 5 risks were present, giving 27.1 times greater risk for growth than a tumor with no risk factors [Table 4].

In 2009, Shields et al.[16] after a retrospective analysis of 2514 patients, further modified their mnemonic to include 'acoustic hollowness' on ultrasound and "halo" surrounding the tumor. In their study of 408 nevi with ultrasound hollowness, 25% showed growth into melanoma compared to 4% tumors that showed growth "without hollowness." The COMS also reported low to medium internal reflectivity, often compatible with acoustic hollowness on B scans, in 88% of choroidal melanomas; further strengthening its role in diagnosis.[17] The halo nevus is a pigmented choroidal nevus surrounded by a halo or a circular band of depigmentation. The absence of such a halo around the lesion has been found to be associated with tumor growth (The halo phenomenon can be found with dysplastic nevus and even with melanoma). In their study, the presence of halo suggested nevus stability. The modified mnemonic proposed was "to find small ocular melanoma using helpful hints daily" which stands for:

- T-Thickness greater than 2 mm
- F-Subretinal fluid
- S-Symptoms
- O-Presence of orange pigment
- M-Margin within 3 mm of the disc
- H-Ultrasound hollowness
- H-Halo absent and
- D-Drusen absent.

They reported that the median Hazard ratio (HR) for growth of nevus into melanoma with 1 or 2 risk factors was 3; for those with 3 or 4 factors, 5; for 5-6 factors, 9; and for all 7 factors, 21 [Table 5]. The highest HR found was 31 for the combination of the following factors: Symptoms (flashing and floaters), the orange pigment, margin near disc, ultrasonographic hollowness, and halo absence. It was recommended that those with 1 or 2 features should be monitored every 4-6 months. Nevi with 3 or more features should be evaluated at an experienced center for management alternatives and possible treatment owing to the high risk of ultimate growth.

Newer imaging techniques

Newer imaging modalities such as enhanced depth imaging-optical coherence tomography (EDI-OCT) and Fundus Autoflouroscence are fast gaining popularity as they are capable of detecting very early changes in a melanocytic lesion harboring malignancy.

Enhanced depth imaging optical coherence tomography

Enhanced depth imaging allows for a detailed structural evaluation of a choroidal pathology. Shields et al.[18] compared the EDI-OCT features of similar sized melanoma with nevi and reported that a melanoma shows characteristic features which include increased tumor thickness, SRF, subretinal lipofuscin deposition, and retinal irregularities, including shaggy photoreceptors [Fig. 1f and i].

Fundus autofluorescence

Fundus autofluorescence (FAF) has been used by several authors to investigate the amount of lipofuscin in the retinal pigment epithelium (RPE). Shields et al.[19] after their study on 51 eyes with small choroidal melanoma, concluded that melanomas show slight intrinsic hyperautofluorescence, the brightness of which increases with pigmented tumors, larger tumors, and with associated RPE disruption [Fig. 1e and h]. They also found that overlying orange pigment showed remarkably bright hyperautofluorescence. Gunduz et al.[20] classified the FAF patterns of choroidal melanocytic lesions as patchy or diffuse. The patchy pattern was defined as the presence of distinct areas of increased FAF between areas of normal autofluorescence. The diffuse pattern was characterized by the presence of increased FAF with indistinct borders over a larger part (>50%) of the tumor in the absence of such intervening areas. They found that choroidal melanomas presented with either a diffuse or patchy pattern, whereas choroidal nevi demonstrated only the patchy pattern. Lavinsky et al.[21] found that choroidal melanomas have a pattern of confluent hyper-auto-fluorescence, nevi, on the other hand, do not have such characteristic hyper-auto-fluorescent features. They, thus concluded that autofluorescence is a useful noninvasive tool to assess lipofuscin in pigmented choroidal lesions, which may contribute to the diagnosis of malignancy.

Table 4: Risk factors predicting tumor growth of a suspected small uveal melanoma (Shields et al.)

Risk factors/ present	RR for growth	Documented tumor growth %
0	1	4
1	1.6-2.3	30-41
2	2.8-5	33-58
3	5.5-9.6	36-63
4	11.6-17.3	39-62
5	27.1	56

Tumor thickness >2 mm, subretinal fluid, symptoms, the orange pigment, margin near optic disc). RR: Relative risk

Table 5: Management guidelines of melanocytic uveal lesion according to the presence of risk factors (Shields et al.)

Number of risk factors	Hazard ratio	Management
1-2	3	Monitor every 4-6 months
3-4	5	Refer to an experienced centre for ocular oncology evaluation
5-6	9	Refer to ocular oncology centre for further management
7 or more	21	Urgent referral to ocular oncology centre

Tumor thickness >2 mm, subretinal fluid, symptoms, the orange pigment, margin near optic disc, ultrasound hollowness, surrounding halo, absence of drusen

Risk factors for metastasis from melanoma

Clinical risk factors

Tumor size has been a hallmark to predict prognosis of the disease. The COMS defined choroidal nevi as any melanocytic choroidal lesion that is <5 mm in the largest basal dimension and is <1 mm in height. A choroidal melanoma is defined as *small* if it measures 3 mm or less in apical height and largest basal diameter of 5.0-16.0 mm, as medium-sized if 3–8 mm in apical height and a basal diameter of not more than 16.0 mm, and as large if >8 mm in apical height or a basal diameter more than 16.0 mm, when the apical height is at least 2.0 mm.[13] Large tumors are associated with poor prognosis and higher metastatic rates. A comparative analysis of the 5-year survival rates after enucleation for differently sized uveal melanomas has indicated that it was 84% for small, 68% for medium-sized, and 47% for large tumors.[22] Shields *et al.* in their study on 8033 patients indicated that increased tumor thickness increases the risk for metastasis. They reported that each increase millimeter in melanoma thickness imparts 5% increased risk for metastatic disease.[23] Other clinical features found to be predictive of metastasis in their analysis were increasing age (HR = 1.13), ciliary body location (HR = 1.68), brown tumor (HR = 1.41) and the presence of SRF (HR = 1.28), intraocular hemorrhage (HR = 1.22) or extraocular extension (HR = 1.41).

Along with tumor thickness, the greatest basal diameter of the tumor also has an impact on patient prognosis. This is of particular relevance in cases with diffuse melanoma in which growth is seen more in a horizontal than vertical direction. Diffuse melanoma has been defined as a tumor with thickness <20% of the tumor base.[24] Damato and Coupland[25] found that metastatic deaths correlated with tumor diameter and found this feature to be a valuable predictor of survival in a cohort of 1,776 patients with uveal melanoma. In a subset analysis of 1751 patients with small melanoma of 3 mm or less in thickness, Shields *et al.*[26] compared prognosis based on diffuse versus nondiffuse configuration. They proved statistically that diffuse choroidal melanoma had a greater risk of metastasis and death. They found that an increase in basal dimension of the tumor by 5 mm increases the risk of metastasis by 5.6 times. Melanocytosis is another risk factor for uveal melanoma development and uveal metastasis. Melanocytosis involving eyelid, sclera or uvea is present in 3% of patients with uveal melanoma. Shields *et al.*[27] studied 7872 patients with uveal melanoma and concluded that the presence of oculodermal melanocytosis doubles the risk for metastasis in melanoma patients when compared with those with no melanocytosis.

Histopathological risk factors

Histopathological features are the gold standard for diagnosing ocular melanoma and differentiating it from a nevus. Following histopathological features have been associated with a poorer prognosis in a melanocytic lesion.

Cell type

The relationship between cell type and prognosis was suspected for nearly a century ago by authors such as Fuchs,[28] Knapp,[29] and Jackson.[30] It was Colonel George Callender in 1931[31] who first proposed a classification for choroidal melanomas. He classified them on the basis of cellular morphology of the tumor into six types, that is, spindle A, spindle B, fascicular, epithelioid, mixed, and necrotic. The latter three were

associated with a poorer prognosis. This classification was subsequently modified by McLean and associates[32] at the Armed Forces Institute of Pathology (AFIP) in 1983 to consist of:
- Spindle cell nevus
- Spindle cell malignant melanoma
- Mixed cell melanoma, and
- Epithelioid cell melanoma.

Using the AFIP modification of the Callender classification, spindle cell tumors carry the best prognosis and epithelioid cell tumors the worst.

Location

Juxtapapillary placed and more anteriorly placed tumors are more likely to be of epitheloid cell and are also more likely to metastasize and progress.[33]

Growth pattern

Diffuse growth pattern has been shown to be associated with a higher incidence of extraocular extensions and higher metastatic potential.[33]

Mitotic figures

Presence of mitotic figures is highly suggestive of malignancy and is a well-known risk factor for metastasis.[33]

Pigmentation

Heavy pigmentation has been found to be associated with epithelioid cell type and larger sized tumors. Increased pigmentation of the tumor is also associated with necrosis and the presence of macrophages, which increases the risk of malignancy.[33]

Necrosis and inflammatory components

The presence of necrosis and inflammatory components within the lesion is associated more with a melanoma.[33]

Vascularity

A choroidal melanoma shows more prominent vasculature and the vascular prominence is often associated with epitheloid cell type and larger size of the tumor.[33]

Molecular risk factors

Micro-metastasis of uveal melanoma is known to occur even prior to primary treatment, which explains substantial rate of metastasis despite treatment.[34] Such metastasis can remain dormant for a prolonged period of time, before becoming clinically detectable.[35] Identifying patients who are at high-risk of harboring undetectable micro-metastases is a challenging but rewarding task. The dissemination of tumor cells into the blood circulation occurs due to lack of lymphatics in the uveal tract. Hematological markers may, therefore, be useful for the detection of distant metastases. This rationale has led for identification of potential molecular markers for the early detection of disseminated tumor cells.

Tyrosinase m-RNA

Serum tyrosinase m-RNA levels have been shown to be increased in patients with primary uveal melanoma. Tyrosinase is an enzyme involved in the synthesis of melanin by melanocytes and melanoma cells. Tyrosinase m-RNA can be used for the indirect quantification of circulating tumor cells, and it has been shown that they even correlate with the size of the primary tumor.[36]

Vascular endothelial growth factor

Overexpression of vascular endothelial growth factor (VEGF) in melanoma cases is a well-documented fact. It originates from abnormal new vessels within the tumor and hypoxia induced by the irregular blood flow. It is proposed that this overexpression is associated with a proliferative stage of the tumor with metastatic potential.[37,38] Levels of VEGF have been associated with the metastatic potential of uveal melanoma,[39] and serum levels are increased in the presence of micrometastases, and they parallel the extent of liver disease.[38,39]

Hepatocyte growth factor

Hepatocyte growth factor and its receptor c-Met have an important role in the growth of cells in the liver. Increased levels of c-Met in uveal melanoma have been shown to be associated with a high risk of metastatic potential.[40]

Insulin-like growth factor-1

Insulin-like growth factor-1 (IGF-1) is also produced in the liver. IGF-1 binds to IGF-1R, a surface membrane glycoprotein. Expression of IGF-1R has been associated with a worse prognosis in uveal melanoma.[41] Activation of IGF-1R by binding of circulating IGF-1 increases cell proliferation, prevents apoptosis and is important for integrin adhesion to the extracellular matrix and invasion of basement membranes.[42] Hence in the presence of metastatic disease, serum IGF-1 levels fall.[43] The co-expression of IGF-1 and c-met in uveal melanoma samples is highly predictive of metastasis.[44]

Despite the promising role of serum molecular markers in determining metastatic disease at a subclinical level, their application in metastatic surveillance is limited as there is a wide variability in the normal range of values within the population.

Cytological risk factors

The advances in treatment for choroidal melanoma have allowed for more and more patients to receive conservative treatment. This has one downside as it precludes obtaining a sample for evaluation of prognostic markers. Naus *et al.*[45] first reported that fine-needle aspiration biopsy (FNAB) could be reliably used to sample tumors for genetic testing of uveal melanoma. In 2007, Shields *et al.*[46] demonstrated in 140 consecutive eyes with choroidal melanoma that tissue sample obtained by FNAB immediately before plaque radiotherapy provides adequate DNA for genetic analysis of uveal melanoma using microsatellite assay.

Chromosomal alterations

Prescher *et al.*[47] were the first to describe the chromosome alterations seen specifically in uveal melanoma. The major chromosome alterations have been described in chromosomes 3, 6, 8, and 11.[48] Interestingly, these chromosomal alterations are significantly correlated with the clinical high-risk factors for metastasis in uveal melanoma such as tumor size at diagnosis and epithelioid cell histology.[48] Loss of one copy of chromosome 3 in tumor cell is seen in nearly half of choroidal melanomas and is a risk factor most strongly associated with metastatic death. Monosomy 3 is associated with a 5-year survival of approximately 50%, whereas disomy 3 has been reported to predict 100% survival.[49] Shields *et al.* in their study on 500 melanoma cases concluded that patients with uveal melanoma demonstrating complete monosomy 3 have substantially poorer prognosis at 3 years than those with partial monosomy 3 or disomy 3.[50]

Using gene expression profiling, melanomas have been categorized into two groups:
- Class I denotes tumors with two copies of chromosome 3 (disomy 3) and other beneficial chromosome changes including gain in chromosome 6p
- Class II denotes tumors with only one copy of chromosome 3 (monosomy 3) and other deleterious chromosome changes including gain of chromosome 8p and/or isochromosome 8p.

It is believed that as the tumor undergoes subsequent growth it either, gains a fragment of chromosome 6p and becomes a less aggressive Class I melanoma or it loses a copy of chromosome 3 and develops into a Class II melanoma with high metastatic potential. Class II tumors have a greater chromosomal aneuploidy and a significantly different proliferative capacity as indicated by the expression of Ki-67 antigen.[51] Patients with Class II tumors need increased metastatic surveillance and entry into adjuvant treatment trials.

Gene alterations

Mutations in genes *GNAQ* and *GNA11* have been associated with the development of uveal melanoma.[44] *GNAQ* and *GNA11* have overlapping functions in melanocytes, and both genes up-regulate the MAP kinase pathway when constitutively active. Nearly 83.0% of uveal melanomas have been found to have a constitutively active mutation in either *GNAQ* or *GNA11*, suggesting that activation of the Gαq–Gα11 pathway is the predominant route to the development of uveal melanoma. *GNAQ* and *GNA11* mutations at codon 209 were encountered in 21.7% and 56.5% of metastatic uveal melanoma samples, respectively.[52] In the same study, *GNA11* mutations were more common in locally advanced tumors and tumors of the ciliochoroidal region. In addition, no association was found with chromosome status reinforcing the notion that these mutations are an early pathogenetic event.[53] The various risk factors for metastasis from melanoma are summarized in Table 3.

Summary

The importance of early identification of a potential metastatic melanoma and the underlying prognostic implications cannot be understated. By combining the various clinical features of the disease with its histopathological, molecular, and cytological features, it is now possible to identify the tumor early in the disease process. Although the role of a good clinical evaluation cannot be underestimated, it is advisable to evaluate the various imaging and molecular and cytological features of small melanocytic choroidal tumors to improve our accuracy to diagnose and provide more favorable prognosis to the patient.

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