CLINICO MORPHOLOGICAL EVALUATION OF MELASMA AND OTHER HYPERPIGMENTARY DISORDERS OF SKIN

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Abstract:

Objectives: To compare clinical presentation and histopathological features of melasma with other hyperpigmentary disorders of skin

Study design: Prospective, observational study

Place and duration of study: outpatient department of Dermatology and Pathology Department, Isra University Hospital Hyderabad from July 2014 - December 2014

Subjects and Methods: Total 87 subjects were selected, 43 having melasma and 44 were with other skin hyperpigmentary disorders. Detailed clinical examination was performed prior to the biopsy to mark the biopsy site. A single strand or in some cases >one punch biopsies of skin were obtained and sent for histopathological evaluation. All the clinical and histological data was entered in Proforma.

Results: Mean age of study subjects was 39.51± 9.32 and 39.50± 9.21 years in melasma and other skin hyperpigmentary disorders respectively. Female population predominated in the present study 49(56.3%) were female. Common presenting features were the hyperpigmentation, skin peeling, itching, erythema and telangiectasia. Hyperpigmentation, itching and erythema were the common presenting complaints. Telangiectasia was noted in 3 male in melasma and 1 female in other skin hyperpigmentary disorders respectively. Female population predominated in the present study 49(56.3%) were female. Common presenting features were the hyperpigmentation, skin peeling, itching, erythema and telangiectasia. Hyperpigmentation, itching and erythema were the common presenting complaints. Telangiectasia was noted in 3 male in melasma and 1 female in other skin hyperpigmentary disorders. Of 43 melasma patients (Group A), the hyperkeratosis was noted in 3 male patients only (\(X^2=48, p=0.0001\)). Parakeratosis and acanthosis were noted in female melasma patients. While hyperkeratosis, parakeratosis and acanthosis were noted in female as well as in male patients of other hyperpigmentary disorders (Group B) (\(X^2=38, p=0.0001\)).

Conclusion: The histopathological findings suggested no major differences in the melasma and other hyperpigmentary disorders.

Keywords: Melasma, Hyperpigmentary skin lesions, Histopathological examination

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INTRODUCTION:
The melanocytes are pigment producing cells of skin which reside in the stratum basale of skin. They are derived from the neural crest cells in the early embryonic period. They migrate to their postnatal anatomical position in various parts of body including the basal layers of epidermis. Inherent to skin, melanocytes produce pigments containing organelles to upper layers of keratinocytes. Melanocytes have enzyme systems which convert tyrosine into a brown black pigment called melanin giving skin its normal hue. Melanocytes once stimulated by physical, chemical, hormonal or any other type of stimulus, produce melanosomes in quantities sufficient to produce skin hyperpigmentation. The sunlight is one of powerful stimulus which stimulates the melanocytes and causes skin pigmentation. (1) The number of melanocytes in skin is found similar in all human races, but the quantity of melanosomes varies. In some races, like Negroes, larger melanosomes give skin a visibly darker complexion. (2, 3) Skin hyperpigmentary lesions of multiple types and include; melasma, solar Lentigines, nevi, macules, café-au-lait spots, ephelides (freckles), etc. Post inflammatory hyperpigmentation also occurs in conditions such as surgical trauma, physical trauma, acne, atopic dermatitis, contact dermatitis, lichen planus, psoriasis, drug eruptions, and etc.(1)During clinical approach to skin hyperpigmentation, one must considers about an increase in melanocytes, increase in melanin pigment production, and/or some other pigment, endogenous or exogenous, which is being deposited in skin.(4) Patient clinical history and physical examination provide clues to the underlying problem causing hyperpigmentation. Onset of lesion is of clinical importance; as neurofibromatosis is a problem causing hyperpigmentation. Onset of lesion may be determined easily during patient’s history. (5, 6) Lesions of Lentigines, ephelides and neurofibromatosis may be diagnosed by the site, size and by their number. Distribution of skin changes helps in differentiating melasma from acanthosis nigricans. Border, color and characteristics of a lesion typically guide to the diagnosis. (7) The lesions of melasma typically look brown, light brown or gray pigmented with macules over the face and or forearms. The facial lesions may be centrofacial-63%, malar 21% and 16% mandibular. Melasma lesions are usually idiopathic, or associated with oral contraceptive use and pregnancy. Phenytoin is also suggested as etiological agent of melasma. Preventive measures and treatment include sunscreen use, and drugs such as retinoids, topical steroids, hydroquinone and glycolic acid peels. For dermal lesions, intense pulsed light therapy and laser are also useful. (4, 6) The characteristic features of solar Lentigines are that they look light yellow, or dark brown, well-circumscribed, and/or variegated in color. They vary in size from 1 to 3 cm macules on the face, forearms, hands, back, shins and or chest. Exposure to ultraviolet light either acute or chronic exposure, is implicated in the etiopathogenesis. Chemical peeling, retinoids, hydroquinone, laser therapy, and or cryotherapy are used in their management. (5) The color of ephelides is red or tan to light brown with sharp defined margins. The average size is 1-2 millimeter. Ephelides appear typically during childhood. Typical sites are the face, neck, arms, chest and legs. Ephelides arise in skin types I and II when exposed to sun light. Treatment is usually not needed for the lesions and they fade in the winter season. Lentigines are tan, brown, or black macules of 2 to 20 millimeter diameter which may arise anywhere on the body. (5, 6) “Café-au-lait” macules are lesions of tan to brown color, epidermal in origin. Lesions measure 1-20 cm, and may be present by birth or appear during early teenage. Trunk is the characteristic site of “Café-au-lait” but may be present anywhere in body. “Café-au-lait” spots arise from accelerated melamin synthesis from melanocytes and keratinocytes of basal epidermal layers. “Café-au-lait” spots are treated by surgery, laser and cosmetic therapy (8). Addison’s disease, thyrotoxicosis and hemochromatosis are causes of diffuse skin hyperpigmentation. Drug may also cause diffuse skin hyper pigmentation. (6, 7) Phototoxic reactions is also cause of skin hyperpigmentation; as may result from use of topical or systemic drug or from a contact with typical plants, foods and exposed to sun light called the phytophotodermatitis. Seborrhea exists as localized, hyper pigmented, hyperplasic and benign lesions, which mimic “melanoma”. Acanthosis nigricans are brown, or velvety brown, streaks and appear as verrucous or papilloma lesions. Acanthosis nigricans is associated with obesity and insulin resistance. (6, 7) Tinea versicolor usually occurs after adolescence, and rarely occurring before this age. Tenia versicolor occurs once production of sebum begins, in particular over the skin of anterior trunk and the back. (9, 10) Physical and chemical trauma, skin injury and various dermatoses may result in post-inflammatory hyper-pigmentation or hypo-pigmentation in any age group (6). As the skin pigmentation is of cosmetic purposes, hence there is need to study melasma and
other Pigmentary disorders. The present study is proposed to compare the clinical presentation and histopathological features of melasma and other hyperpigmentary skin lesions. Hyper pigmented lesions especially the melasma is a common problem in Asia and other parts of the world. Studies relevant to these hyper pigmented disorders are lacking in Pakistan. Keeping these facts in mind the present study is conducted to evaluate the various modes of clinical presentation and morphological features of melasma and other hyperpigmentary disorders in Hyderabad. The results obtained from the study will help the clinicians in understanding clinical presentation and management of the hyperpigmentary disorders.

METHODOLOGY:
This prospective, observational study was conducted at the outpatient department of Dermatology Department Isra University Hospital Hyderabad. Study duration was 6 months from July 2014 - December 2014. All the patients with skin hyperpigmentation, age 20 to 60 years either gender were included. All the patients receiving treatment for hyperpigmentation currently or within past 3 months, pregnancy, oral contraceptive pill therapy, hormone replacement therapy, diabetes mellitus, keloid tendency and drug intake any suspected drug causing skin hyperpigmentation. All the patients were divided in two groups
- Group A (n=43) - melasma patients
- Group B (n=44) - other skin hyperpigmentary disorders

Detailed clinical examination was performed prior to the biopsy to mark the biopsy site. Aspirin and non-steroidal anti-inflammatory drugs were stopped at least 5 days before biopsy. A single strand or in some cases >one punch biopsies of skin (from lesional area) were obtained with control (single strand of skin biopsy from normal skin) and sent for histopathological evaluation. All the clinical and histological data were entered in Proforma designed for the study.

Thorough gross examination of tissues was done to check the number of skin biopsies and they were noted according to Proforma.

Steps of Tissue Processing: For the processing: manual procedure was adopted which included following steps.

Fixation: Fixation was done by fixators. Skin biopsy specimens were preserved in 10% formalin. 10% formalin was made by diluting 40% formaldehyde in water. Thus in 10% formalin the actual concentration of formaldehyde was 4%.

Dehydration: Dehydration was done to dehydrate the tissue and to facilitate sectioning. Dehydration of the sections from skin biopsy was done by passing them through the ascending order of alcohol as given below:
- Alcohol 80% 01 hour
- Alcohol 95% 02 hours
- Alcohol 95% 01 hours
- Absolute alcohol 100% 01 hour
- Absolute alcohol 100% 01 hour
- Absolute alcohol 100% 01 hour

Clearing: This was carried out by using Xylene. First tissues were put in 50% V/V solution of alcohol – Xylene and then in pure xylene.
- 50% alcohol – xylene V/V
  - 01 hour
- Pure xylene
  - 02 hours

Impregnation: This step was carried out by putting tissue into paraffin wax at melting point of 58°C.
- Melted Paraffin wax I
  - 02 hour
- Melted Paraffin wax II
  - 02 hour
- Melted Paraffin wax III
  - 02 hour

Embedding: The tissues were embedded in blocks of paraffin wax by using L shaped molds or plastic molds and were allowed to solidify and frozen to hard in freezer then blocks were formed.

Sectioning: The paraffin wax blocks were sectioned in to 3 µ thick sections by using rotatory microtome, average 2-3 slides were prepared from each block by taking ribbons of tissues containing 2-3 section on each slide.

Fixation of Tissue over the slide: The sections were put in tissue floating bath at the temperature of 37°C then taking up on the slides layered with egg albumin. Tissue sections were fixed on slides by putting them in incubator at temperature about 37°C for about 2-3 hours.

Removal of Wax: De-waxing was done by addition of xylene because wax is soluble in xylene.

Rehydration: Rehydration was done by adding absolute alcohol in xylene and then decreased alcohol in descending order like; 100%----95%----90%----80%----70% and distilled water.

Staining of Sections: Then sections were stained with routine Hematoxylin and Eosin staining.
- The sections were put in Harris hematoxylin for 5 minutes.
- The sections were washed in running tap water for 5 minutes.
- These were differentiated in 1% acid alcohol (1% HCl in 70% alcohol) for 5 minutes.
- The sections were washed in tap water for 5 minutes or even less.
- The sections were stained for eosin for 10 minutes.
After staining of slides, alcohol in ascending order i.e., 70%, 80%, 95%, and 100% then were put it in xylene for removal of alcohol. Mounting was done by putting one drop of Canada balsam or DPX (Di-N-Butyle Phthalate in xylene) then we put the cover slip on

The findings of tissue biopsies were compared for histopathological finding found in melasma with other hyperpigmentary disorders, as well as Perivascular inflammatory cells infiltration and melanocytes infiltration at epidermal and dermal level. Grading of Perivascular inflammatory infiltrate was done according to Perivascular inflammatory infiltration and graded as grade:1 = (none 0%), grad:2 = Mild (< 50%), grad:3 = Moderate (50%), grad:4 = Severe (> 50%). Grading of hyperpigmentation in skin biopsy was done according to Number of melanophages (epidermal, dermal & epidermo-dermal level) and graded as grade:1 = (none 0%), grad:2 = Mild (< 50%), grad:3 = Moderate (50%), grad:4 = Severe (> 50%). Sample size for the study was calculated by the following formula:

\[ n = \frac{(Z1 - \alpha/2)^2 \times p \times (1-p)}{d^2} \]

Where
\( n \) is the sample size, \( (Z1 - \alpha/2) \) is the probability level or confidence level at 95% (1.96). \( p \) is the probability of an event (the prevalence of hyperpigmentary disorders is 65.7%), \( 1-p \) is the probability of an event that is not occurring (in this case it is \( 1-p = 1 - 0.65 = 0.35 \)) and \( d \) is the margin of sampling error (it is taken at 10%)

\[ n = \frac{(1.96)^2 \times 0.65 \times 0.35}{(0.10)^2} \]

\[ n = 3.84 \times 0.2275 \]

\[ n = 0.8736 \]

\[ n = 87.36 \]

\[ n = 87 \]

The data was analyzed on SPSS version 21.0 for windows release (IBM, incorporation, USA). Continuous and categorical variables were analyzed using students t-test and chi-square test. Continuous variables were tabulated as mean ±SD and categorical variables as frequency and %. Pearson’s correlation was used for continuous variable. Microsoft excel was used for graphing purpose. Data was presented as tables, charts and graphs. P-value ≤ 0.05 was defined as significant.

RESULTS:
The present study was conducted at the Department of Pathology and Dermatology, Isra University. Objective of the study was to evaluate clinical presentation histopathological features of melasma in comparison to other skin hyperpigmentary disorders in a selected region of Sindh province.

Mean ± SD age of study subjects was noted as 39.51± 9.32 and 39.50± 9.21 years in melasma and other skin hyperpigmentary disorders respectively (table IV-1 and graph IV-1). Mean ± SD age according to gender is shown in graph IV-2. Age distribution as different categories is shown in table IV-3. There were 29 (33.3%) under 4th decade and 58 (66.6%) above 4th decade (p=0.061). Graphical presentation of age distribution is shown in graph IV-1 to IV-3.

Female population predominated in the present study. 38 (42.5%) were male and 49 (56.3%) were female (X²=14.4, p=0.01). Gender distribution is shown in table IV-2 and graphs VI-4 to IV-5.

Presenting clinical features in total study population are summarized in table IV-3 and graphs IV-6. Common presenting features were the hyperpigmentation, skin peeling, itching, erythema and telangiectasia (X²=40.01, p≤0.01). Hyperpigmentation, itching and erythema were the common presenting complaints. Telangiectasia were noted in 3 male in melasma and 1 female in other skin hyperpigmentary disorders as shown in table IV-3 and graph IV-6 (X²=49.01, p≤0.01).

Clinical signs are shown in figure IV-1 through IV-6, showing male and female of melasma and other skin hyper Pigmentary disorders. Site, size and skin fragments characteristics of both groups are shown in table IV-4 to IV-6. Most common sites of pigmentation were the face and cheeks as shown in table IV-4 and graph IV-7 and IV-8. Skin hyper pigmentation was most commonly observed on the face. Size of skin lesions was categorized as < 1 cm and 1 – 3 cm are shown in table IV-5. Most of the skin lesions size was < 1 centimeter (X²=87, p=0.0001). Skin lesions fragment characteristics are shown in table IV-6 and graph IV-10. > 1 Linear fragment skin lesions were noted in both melasma and other skin hyperpigmentary disorders as shown in table IV-6 and graph VI-10.

Gross Macro-Photography
Macro photography as shown in figure IV-1 through
IV-6, shows pictures of male and female of group A (melasma) and group B (other skin hyper Pigmentary disorder) patients.

**Histopathological Examination**

**Microscopic findings:**
Microscopic findings of skin lesions are shown in table IV-7 and graph IV-11 and IV-12. Of 43 melasma patients (Group A), the hyperkeratosis was noted in 3 male patients only ($X^2$=48, $p=0.0001$) with no parakeratosis and acanthosis, also there were no any case of hyperkeratosis, parakeratosis and acanthosis found in female melasma patients. While hyperkeratosis, parakeratosis and acanthosis were noted in female as well as in male patients of other hypopigmentary disorders (Group B) ($X^2$=38, $p=0.0001$).

**Perivascular inflammatory cell infiltrate:**
Grading of Perivascular inflammatory cell infiltrate is shown in table IV-8 and graph IV-13. Melasma group (group A) showed 4 patients in grade 2, 39 in grade 1 and none in grade 3 and 4. While group B (other hypopigmentary disorders), 44 patients were of grade 2.

**Number of Melanophages:**
Numbers of melanophages in epidermal, dermal and epidermo-dermal junction level are shown in table IV-9, graph IV-14 and IV-15.

- Of total 43 melasma patients (group A):
  - 30 showed grade 2 – epidermal melanophages,
  - 6 showed grade 3 – epidermal melanophages,
  - 1 showed grade 4 – epidermal melanophages and
  - 6 showed grade 4 – epidermo-dermal melanophages (as shown in table IV-9, graph IV-14 and IV-15).

- Of total 44 - other skin hyperpigmentary patients (group B):
  - 32 showed grade 1 – epidermal melanophages,
  - 10 showed grade 2 – epidermal melanophages,
  - 2 showed grade 3 – epidermal melanophages.

Major statistical differences were observed in number of melanophages in group A and B patients as shown in table IV-9, graph IV-14 and IV-15.

**Micro photography of skin biopsies - Histopathological Examination**
Micro photography of melasma (group A) patients is shown in photomicrographs IV-1 to IV-5. Photomicrograph IV-2 shows epidermal melasma of grade 2 with <50% of pigmentation of skin. Photomicrograph IV-4 shows epidermal melanophages of grade 4 with >50% of pigmentation. Photomicrograph IV-5 shows epidermal melanophages of grade 3 i.e. 50% of pigmentation.

<table>
<thead>
<tr>
<th>Table 1. Age and Gender distribution (years) of study population (n=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (Mean ±SD)</strong></td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>39.51± 9.32</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Presenting clinical features of study population (n=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Presenting features</strong></td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Hyper pigmentation</td>
</tr>
<tr>
<td>Peeling</td>
</tr>
<tr>
<td>Itching</td>
</tr>
<tr>
<td>Erythema</td>
</tr>
<tr>
<td>Telangiectasia</td>
</tr>
</tbody>
</table>
### Table IV-3. Site of lesions of study population

(n=87)

<table>
<thead>
<tr>
<th>SITE AND SIZE</th>
<th>GROUP A (n=43)</th>
<th>GROUP B (n=44)</th>
<th>X²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>SITE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Face</td>
<td>10</td>
<td>33</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>Cheeks</td>
<td>10</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Forehead</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Upper lip</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nose</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Feet</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Arms &amp; Chest</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Chest</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Hands</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Scalp</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Arms</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>SIZE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 cm</td>
<td>28</td>
<td>20</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>1-3 cm</td>
<td>15</td>
<td>13</td>
<td>18</td>
<td>6</td>
</tr>
</tbody>
</table>

### Table IV-4. Microscopic findings AND Skin lesion fragment characteristics of study population

(n=87)

<table>
<thead>
<tr>
<th>SKIN LESION FRAGMENT</th>
<th>GROUP A (n=43)</th>
<th>GROUP B (n=44)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; Single Linear fragment</td>
<td>3</td>
<td>8</td>
<td>0.0001</td>
</tr>
<tr>
<td>&gt; &gt; 1 linear fragments</td>
<td>7</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; Single Linear fragment</td>
<td>10</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>&gt; &gt; 1 linear fragments</td>
<td>23</td>
<td>11</td>
<td></td>
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### Microscopic findings

<table>
<thead>
<tr>
<th>Male</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Hyperkeratosis</td>
<td>3</td>
<td>28</td>
<td>0.0001</td>
</tr>
<tr>
<td>Parakeratosis</td>
<td>0</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Acanthosis</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Female</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperkeratosis</td>
<td>0</td>
<td>16</td>
<td>0.0001</td>
</tr>
<tr>
<td>Parakeratosis</td>
<td>0</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Acanthosis</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Grades</td>
<td>Group A (n=43)</td>
<td>Group B (n=44)</td>
<td>$\chi^2$</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>Grade 1 (None)</td>
<td>39</td>
<td>0</td>
<td>211.0</td>
</tr>
<tr>
<td>Grade 2 (&lt;50%)</td>
<td>4</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Grade 3 (50%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grade 4 (&gt;50%)</td>
<td>0</td>
<td>0</td>
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Table IV-6. Number of Melanophages at Epidermal, Dermal and Epidermo-dermal level

<table>
<thead>
<tr>
<th>Melanophages</th>
<th>Site</th>
<th>Group A (n=43)</th>
<th>Group B (n=44)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1 (None)</td>
<td>Epidermal</td>
<td>0</td>
<td>32</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Dermal</td>
<td>0</td>
<td>0</td>
<td>$\chi^2 = 37.0$</td>
</tr>
<tr>
<td></td>
<td>Epi+Dermal</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grade 2 (&lt;50%)</td>
<td>Epidermal</td>
<td>30</td>
<td>10</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Dermal</td>
<td>0</td>
<td>0</td>
<td>$\chi^2 = 63.0$</td>
</tr>
<tr>
<td></td>
<td>Epi+Dermal</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grade 3 (50%)</td>
<td>Epidermal</td>
<td>6</td>
<td>2</td>
<td>0.0001</td>
</tr>
<tr>
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<td>Dermal</td>
<td>0</td>
<td>0</td>
<td>$\chi^2 = 41.0$</td>
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<td>Epi+Dermal</td>
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<td>Epidermal</td>
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Photographs. IV-1. Pictures of female patients suffering from Melasma
Photographs IV-2. Pictures of female patients suffering from Melasma
Photographs. IV-3. Pictures of female patients suffering from Melasma.
Photographs. IV-4. Pictures of male patients suffering from Melasma.
Drug eruptions
Lichen planus
Post burn hyperpigmentaiton
Drug eruptions
Insect bite
Seborrheic dermatitis
Tinea capitis
Psoriasis
Tinea versicolor
Chronic melanocytic nevi
Viral wart
Eczema

Photographs. IV-5. Pictures of other Pigmentary disorders
Photographs. IV-6. Pictures of other Pigmentary disorders
I. HISTOPATHOLOGICAL FINDINGS OF SKIN BIOPSIES OF MELASMA (GROUP A) PATIENTS:

**Photomicrograph 1.** Histopathological findings of melasma skin biopsy

**Photomicrograph IV-2.** Epidermal melasma showing <50% Pigmentation (grade 2)

**Photomicrograph IV-3.** Hyperkeratosis of noted in melasma
Photomicrograph IV-4. Histopathological findings suggest a grade 4
(Epidermal melanophages>50%) (n=6)

Photomicrograph IV-5. Histopathological findings suggest a
Grade 3 findings (50% epidermal melanophages) (n=6)

II. HISTOPATHOLOGICAL FINDINGS IN OTHER PIGMENTARY DISORDERS (GROUP B):

Photomicrograph IV-6. Histopathological findings of grade 3 epidermal melanophages (n=2 cases of keloids)
Photomicrograph IV-7. Histopathological findings of grade 3 epidermal melanophages (n=2 cases of keloids)

Photomicrograph IV-8. Grade 2 <50% melanophages case of acne (6 cases of acne shows melanophages activity >50% rest of 1 cases has not any melanophages activity but all cases of acne shows grade 2 inflammatory infiltration).

Photomicrograph IV-9. Histopathological findings of acne cases (grade 2 melanophages & grade 2 Perivascular)
Photomicrograph IV-10. Histopathological findings of Grade 1 melanophages & grade 2 Perivascular acne case

Photomicrograph IV-11. Histopathological slide showing hyperkeratosis, parakeratosis and acanthosis (3 cases of acanthosis belong to lichen planus with grade 2 Perivascular infiltration & grade 1 melanophages in lichen planus

Photomicrograph IV-11. Histopathological findings of lichen planus.
Photomicrograph IV-12. Histopathological slide showing Hyperkeratosis and parakeratosis in lichen planus

Photomicrograph IV-13. Histopathological slide showing Prurigonodularis with grade 2 < 50% melanophages activity and grade 2 Perivascular infiltration both cases of grade 3 melanophages belongs to Prurigonodularis.

Photomicrograph IV-14. Histopathological slide showing case of Prurigonodularis with hyperkeratosis parakeratosis grade 2 Perivascular infiltrations and grade 2 melanophages activity.
Photomicrograph IV-15. Histopathological Tinea versicolor (7 cases with grade 1 melanophages and grade 2 Perivascular infiltration)

Photomicrograph IV-16. Histopathological slide of Tinea corporis 10 cases 1 cases shoed grade 2 melanophages& all 10 cases showed grade 2 Perivascular infiltrations with hyperkeratosis.

Photomicrograph IV-17. Histopathological findings of Tinea cruris which shows grade 2 melanophages activity and grade 2 Perivascular infiltration, with hyperkeratosis

Photomicrograph IV-18. Histopathological findings of Tinea cruris which shows grade 2 melanophages activity and grade 2 Perivascular infiltration, with hyperkeratosis
Photomicrograph IV-19. Histopathological findings of Tineacurris which shows grade 2 melanophages activity and grade 2 Perivascular infiltration, with hyperkeratosis

Photomicrograph IV-20. Histopathological findings of Tineapaedis 2 cases and 1 case of tineafacialis and 1 case of Tineacapitis just showed hyperkeratosis, parakeratosis with grade 2 Perivascular infiltrations with grade 1 melanophages activity.

Photomicrograph IV-21. Histopathological findings -Five cases of viral Warts all shows hyperkeratosis with parakeratosis with grade 2 Perivascular infiltration and grade 1 melanophages activity
DISCUSSION:
The present study was conducted at the Department of Pathology and Dermatology, Isra University Hospital, Hyderabad. The study intended to investigate differences of clinical presentation and histopathological examination in melasma patients compared to other skin hyper Pigmentary disorders. To the best of our knowledge, it is the first study ever conducted in the country.

Melasma occurs because of dysfunction of melanogenesis which results in acquired chronic localized skin hyperpigmentation. Melasma occurs symmetrically on the sun exposed parts of human body involving in particular the women in their menacme of age (11).

Melasma originates from its Greek root “melas” that mean “black”, the term typically reveals brown or brownish black hue of the lesion. Chloasma, derived from the Greek root “cloazein” and or Latin root “chloos” means “greenish” is semantically use in the literature (12-14).

Melasma is reported to affect all ethnical and population groups, however, epidemiological data shows a higher prevalence pigmented skin phenotypes as those belonging to East Asians – Chinese, Korean and Japanese, Pakistanis and Indians, Middle Eastern populations, and the Mediterranean-African origin ethnicity. Melasma is common among Hispanic-Americans and Brazilians living in inter-tropical areas, where there are greater chances of sun exposure i.e., UV rays (15-17).

As regards the studies on the histopathological findings and clinical presentation of melasma and other skin hyper pigmentary disorders, a search of medical literature on the Pubmed, Medlip, and Medline showed an absolutely deficient data on the current topic from the Pakistan. Paucity of cited literature on the histopathological spectrum of melasma and other skin hyperpigmentary disorders compelled the researcher to gather the data at our tertiary care hospital.

In present study, the mean ± SD age was noted as 39.51± 9.32 and 39.50± 9.21 years in melasma and other skin hyperpigmentary disorders respectively. Mean ± SD age according to gender is shown in graph IV-2. Age distribution as different categories is shown in table IV-3. There were 29 (33.3%) under 4th decade and 58 (66.6%) above 4th decade (p=0.061). Graphical presentation of age distribution is shown in graph IV-1 to IV-3. The findings of age of present study are in comparison to previous studies (18, 19).

Female population predominated in the present study. 38 (42.5%) were male and 49 (56.3%) were female. The female preponderance has been pointed out in previous studies (18,19), which support the findings of present study, but contrary to a study from India by Sarkar R, et al (20), which has reported 1 out of 5 was a male patients.

A clear female predominance has been reported of 9:1 or 10:1. A study from India has reported less significant prevalence ratio of 6:1, while studies from Brazil and Singapore had reported strong female predominance with a ratio of 39:1 and 21:1 respectively (21-23). In present study, male to female ratio of melasma was 1:3.3 (table IV-2) which is consistent to Indian study but contrary to studies from Brazil and Singapore as mentioned above.

Common presenting features were the hyperpigmentation, skin peeling, itching, erythema and telangiectasia. Hyperpigmentation, itching and erythema were the common presenting complaints. Telangiectasia was noted in 3 male in melasma and 1 female in other skin hyperpigmentary disorders. The findings are parallel to previous studies (18, 19).

Most common sites of pigmentation were the face and cheeks. Skin hyper pigmentation was most commonly observed on the face. Most of the skin lesions size was < 1 centimeter. >1inear fragment skin lesions were noted in both melasma and other skin hyperpigmentary disorders. The findings are in keeping to previous studies (18, 19) but in contrast to others (21-23).

For understanding the pathogenesis of hyperpigmentation in melasma, physiological functioning of melanocytes and their contribution to skin color phenotypes, or skin coloration in genetically determined skin which becomes altered to facultative skin coloration by influence of environmental factors such as sun light or inflammation, and hormones needs to be explored (18).

In the present study, of 43 melasma patients (Group A), the hyperkeratosis was noted in 3 male patients only with no parakeratosis and acanthosis were noted in male patients while in female melasma patients no any case of hyperkeratosis, parakeratosis and acanthosis were found. While hyperkeratosis, parakeratosis and acanthosis were noted in female as well as in male patients of other hyperpigmentary disorders (Group B).

In present study perivasculary inflammatory cells infiltration findings in melasma group (group A)
showed 4 patients in grade 2, 39 in grade 1 and none in grade 3 and 4. While group B (other hyperpigmentary disorders), 44 patients were of grade 2, no findings in grade 1, 3 and 4... Numbers of melanophages, of total 43 - melasma patients (group A); 30 showed grade 2 – epidermal melanophages, 6 showed grade 3 – epidermal melanophages, 1 showed grade 4 – epidermal melanophages and 6 showed grade 4 – epidermo-dermal melanophages. While, of - other skin hyperpigmentary patients (group B); 32 showed grade 1 – epidermal melanophages, 10 showed grade 2 – epidermal melanophages, 2 showed grade 3 – epidermal melanophages. The above findings are in consistency to previous studies of Sarvjot et al (18), Sanchez et al (24).

A few comparative studies had been cited on the comparison of melasma and other melanocytic pigmentary disorders. The correlation of melasma with post inflammatory hyper-pigmentation is poorly cited in the medical literature (25).

A case control study from Iran, included 120 skin disorders and reported melasma was more prevalence among cases of freckles, lentigines, ruby angiomas and nevi, this may indicate as marker of risky patients phenotypes (26).

A study from Iran included 200 melasma patients with associated inflammatory acne and control subjects with inflammatory acne without melasma. The study reported 6 times more chances of developing post inflammatory hyper-pigmentation in melasma patients compared to inflammatory acne without melasma. Hence, it is concluded that the melanocytes are over active in melasma patients (27).

A similar finding of post inflammatory hyper pigmentation in melasma patients has been reported in a study from Brazil (21).

Micro photography of melasma (group A) patients is shown in photomicrographs IV-1 to IV-5. Photomicrograph IV-2 shows epidermal melanoma of grade 2 with <50% of pigmentation of skin. Photomicrograph IV-4 shows epidermal melanophages of grade 4 with >50% of pigmentation. Photomicrograph IV-5 shows epidermal melanophages of grade 3 i.e. 50% of pigmentation. The findings are in keeping to previous studies Sanchez et al (24).

Histopathological features suggested an increase in number of melanocytes, melanophages, and melanosomes with pigmentation of epidermal, dermal and epidermo-dermal junctions were noted. These findings are in consistency to previous studies (724, 28, and 29). These previous studies had reported increased number and activity of melanocytes, melanogenesis, and increased transfer and reduced degradation of melanosomes in the keratinized layers of skin which is an essential phenomenon for the pathogenesis of melasma. The findings are consistent to previous studies (24, 28-30)

Sanchez et al (24) had reported a study in which the to-pathological features of 17 melasma patients were evaluated. The skin biopsy was taken by Thiersch graft technique contrary to present study, as we have taken skin punch biopsy. The skin punch biopsy is superior in tissue yielding hence our results are more authentic.

Sanchez et al (24) reported an increase in epidermal number of melanocytes, melanin with basilar vacuopathy but sparse reactions of lymph histiocytic infiltrate.

In present study, more number of melanocytes, melanosomes, melanophages and melanin pigmentation were observed similar to previous studies (24, 28, 29). Previous studies had reported more number of melanocytes and melanin in sun exposed skin of face contrary to other parts of body (24, 28, 29). The findings are highly consistent to present study, as face was the noticeable site of our study population and histopathological findings are in parallel to above studies.

Our study included 43 melasma and 44 patients and found an increase in the melanin with H&E and Masson-Fontana stains in all the epidermal layers. The findings are similar to Sanchez et al (24) and Sarvjot et al (24).

Epidermal thinning with rete ridges flattening were observed in histopathological examination.

In present study, a sparse to mild perivascular inflammatory infiltrate in 60% of patients was noted; the findings are supported by previous studies of Sanchez et al (76) and Sarvjot et al (24).

Grimes et al (28) has also reported a study showing mild perivascular lymphocytic infiltrate in 75% of biopsies. In present study, melasma (group A) showed 4 patients in grade 2, 39 in grade 1 and none in grade 3 and 4. While group B (other hyperpigmentary disorders), 44 patients were of grade 2. Epidermal melanoma of grade 2 was observed with <50% of pigmentation of skin. Photomicrograph IV-4 shows epidermal melanophages of grade 4 with >50% of pigmentation. Photomicrograph IV-5 shows epidermal melanophages of grade 3 i.e. 50% of pigmentation. Our findings are consistent to Grimes
et al (28), Sanchez et al (24) and Sarvjot et al (24).

The histopathological findings of melasma of present study are in confirmation with the previous study of Kang et al (29) from Korea, this previous study reported similar histopathological features of melasma in Korean women using methodologies similar to present study.

In present study, the skin punch biopsies of 2-mm-thick were taken for histopathological examination. The concentration of melanin was significantly increased in epidermis and dermis of melasma patients. The staining intensity and number of melanosomes were increased in epidermal, epidermo-dermal layers and dermis of melasma skin. These findings are highly consistent to previous studies (18, 19, 24).

In melasma hyperpigmentation is largely epidermal under Wood's lamp. However, melasma may, in some cases, present with significant pigmentation of dermis also, which give grayish hue to skin and Wood’s lamp examination fails to demonstrate it (19).

The clinical and histopathological association of increased dermal melanin pigmentation and negative Wood's lamp examination is controversial, Sanchez et al (24) has shown a positive association while Grimes et al (28) denied it. The findings of epidermal pigmentation are consistent to present study, while dermal pigmentation in melasma was not observed in the present study which is similar to Grimes et al (28) but contrary to Sanchez et al (24). However, epidermo-dermal pigmentation in melasma patients was observed in our present study. Dermal pigmentation was not observed in any of patients of 2 groups of present study as shown in table IV-9 and graph IV-14 and IV-15.

It is suggested that the histopathological features of melasma per se are usually subtle and are truly appreciated only when control skin biopsies are available (28-30). The pigmentation is observed mainly in the basel/suprabasal cells as “pigmentary-caps” (28, 29). However, increased melanin pigmentation has been reported in all epidermal layers in a Korean study (81), this supports the findings of present study.

Dermal melanin has been reported by Sanchez et al (24) mainly within the macrophages called as “melanophages”, however, free melanin around vascular plexuses- both superficial and deep, and has been reported. The findings of above study are in absolute contradistinction to present and previous study of Grimes et al (28).

Increased melanocyte activity has been reported in melasma patients using “Mel-5 immuno-staining” (28, 29). It is reportedly varies from an increase in the number of melanocytes to merely enlargement of the melanocytes – as indicated by increased staining and prominent melanocytic dendrites both of which are indicative of increased melanogenesis (28,29). The findings of above studies are in parallel to our present study.

Due to the incomplete understanding of melasma pathogenesis, its treatment essentially aims at blocking sun exposure; which reduces melanin synthesis, transport and transfer of melanosomes, and by reducing the amount of epidermal melanin (31-34). The long-term therapies are essentially necessary as the recurrence rates are very high. However, this matter is beyond the scope of the present research study.

In general, clinical presentation of melasma mimics with other hyper pigmentary disorders. However, the histopathological findings of melasma are similar to other hyper pigmentary skin diseases. The present study has some of its limitations; firstly- small sample size, and secondly– the specific study population, hence the results cannot be generalized to other settings.

Controlled epidemiological studies may support hypotheses for research on pathophysiology, therapeutic strategies, as well as promote primary prevention in risk groups.

CONCLUSION:
Common presenting features were the hyperpigmentation, skin peeling, itching, erythema and telangiectasia in melasma and other hyperpigmentary lesions. Hyperpigmentation, itching and erythema were the common presenting complaints. Telangiectasia’s were noted also. Histopathological findings suggested no major differences in the melasma and other hyperpigmentary disorders. Further studies are recommended to confirm the findings of present study. Treatment options for melasma and other skin hyperpigmentary disorders should be elaborated in in-depth studies.

REFERENCES:


