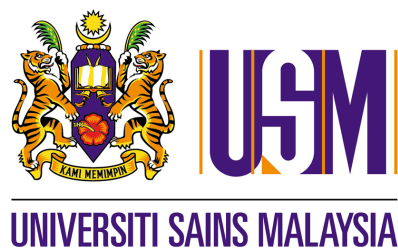


**A CROSS-SECTIONAL STUDY
TO ASSESS PATTERN IN WRINKLE FORMATION
IN DIFFERENT AGE GROUPS
IN FEMALE POPULATION IN KELANTAN**

ZOSIMO KEN L. JIMENO IV
DISSERTATION SUBMITTED IN PARTIAL FULFILMENT
OF THE REQUIREMENT FOR
THE DEGREE OF MASTER OF SURGERY (PLASTIC)



2018

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I would like to extend my utmost gratitude to my family members for their unending support and unfathomable grace, without whom I would not stand where I am now.

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Table of Contents

No	Contents	Page
I	Acknowledgment	ii
II	Table of Contents	iii
III	List of Abbreviation	v
IV	List of Tables	vi
V	List of Figures	vii
VI	Abstrak	viii
VII	Abstract	ix
1	Introduction	
1.1	Literature Review	2
1.1.1	Facial Aging Process	2
1.1.2	Superficial Textural Wrinkling	3
1.1.3	3-Dimensional Topographic Changes	6
1.1.4	Effects of Smoking ad Sun Exposure to Skin Aging	7
1.1.5	Digital Photography in Facial Analysis	8
1.2	Rationale of the Study	9
2	Study Protocol	
	Dissertation Proposal	13
	Summary of Research Proposal	14
2.1	Study Protocol	
2.1.1	Introduction	15
2.1.2	Objectives	22
2.1.3	Methodology	22
2.1.4	Expected Results & Benefits	30
2.1.5	Declaration of Conflict of Interest	30
2.1.6	References	30
2.1.7	Appendices	33
2.1.8	Written Consent	39
2.2	Ethical Approval from Human Research Ethics Committee USM (HREC)	
	Approved with Correction	52
	Approved after Amendment	54
3.	Manuscript	
1)	Title Page	58
2)	Abstract	60
3)	Main Text	62

Introduction	62
Methodology	
Materials and Methods	64
Analysis	66
Results	66
Comparison of Means of Wrinkles between Age Groups	67
Correlation of Glabella, Forehead and Frontal Face Wrinkles	67
Discussion	68
Future Research and Clinical Applications	70
Conclusion	70
Limitations	71
4) Declaration of Conflict of Interest	72
5) References	73
6) Tables	76
7) Figures	78
4 Appendices	
4.1 Test of Data Normality	82
4.1.1 Test of Normality of Glabella Wrinkles data in each Age Group	82
4.1.2 Test of Normality of Forehead Wrinkles data in each Age Group	84
4.1.3 Test of Normality of Frontal Face Wrinkles data in each Age Group	85
4.2 Auxiliary findings from the data gathered	87
4.2.1 Association between Age Group and Secondary Smoking with formation of Facial Wrinkles	87
4.2.1.1 Letter of Acceptance for Oral Presentation in the 18 th ASEAN Congress of Plastic Surgery	90
4.2.1.2 Certificate of Oral Presentation	91
4.2.2 Association between Age Group and Water Intake with formation of Facial Wrinkles	92
4.2.2.1 Letter of Acceptance for Oral Presentation in the 4 th Annual Scientific Meeting of Malaysian Society of Plastic Reconstructive Surgery (MSPRS)	94
4.2.2.2 Certificate of Oral Presentation	95
4.2.3 Association between Age Group and Fitzpatrick Skin Group with formation of Facial Wrinkles	96

4.2.4 Association between Age Group and Body Mass Index with formation of Facial Wrinkles	99
4.2.5 Association between Age Group and Income Group with formation of Facial Wrinkles	101
5 Reference to Journal Submission	102

List of Abbreviation

BMI	Body Mass Index
CPI	Cross Polarisation Image
DSLR	Digital Single-Lens Reflect
FSG	Fitzpatrick Skin Group
PPI	Parallel Polarisation Image

List of Tables

No	Title	Page
Table 1	Fitzpatrick's Classification of Facial Wrinkling (Perioral and Periorbital)	5
Table 2	Modified Fitzpatrick Wrinkle Scale (MFWS)	6
Table 3	Fitzpatrick's Classification of Facial Wrinkling (Perioral and Periorbital)	17
Table 4	Modified Fitzpatrick Wrinkle Scale (MFWS)	18
Table 5	Demographic Data	76
Table 6	Percentage of wrinkles in glabella, forehead and frontal face	77
Table 7	Descriptive Analysis of Glabella Wrinkles in each Age Group	82
Table 8	Normality Test for Glabella Wrinkles Data	83
Table 9	Descriptive Analysis of Forehead Wrinkles in each Age Group	84
Table 10	Normality Test for Forehead Wrinkles Data	85
Table 11	Descriptive Analysis of Frontal Face Wrinkles in each Age Group	85
Table 12	Normality Test for Frontal Face Wrinkles Data	86
Table 13	Smoking and Frontal Face Wrinkle Formation	88
Table 14	Water Intake and Frontal Face Wrinkle Formation	93
Table 15	Mean Frontal Face Wrinkles based on Fitzpatrick Skin Group	96
Table 16	Fitzpatrick Skin Group and Frontal Face Wrinkle Formation	97
Table 17	Body Mass Index (BMI) and Frontal Face Wrinkle Formation	99
Table 18	Monthly Income and Frontal Face Wrinkle Formation	101

List of Figures

No	Title	Page
Figure 1	Digital Photographic Image System, Dermavision.	10
Figure 2	Digital Photographic Image System, Dermavision.	21
Figure 3	Region of interest at the glabella	27
Figure 4	Region of interest at the forehead	27
Figure 5	Region of interest at the frontal face	28
Figure 6	Digital Photographic Image System, Dermavision.	78
Figure 7	Regions of interest in the facial wrinkle study.	79
Figure 8	Example of frontal image capture and selection of area of interest for study of frontal face.	79
Figure 9	Means and standard deviation of wrinkle formation in different areas of study – glabella, forehead and frontal face, according to age groups.	80

Abstrak

Latar Belakang Kedutan muka merangkumi kedutan superfisial dan pergerakan serta garis semula jadi muka, yang mana pembentukannya disumbang oleh faktor-faktor intrinsik dan ekstrinsik. Penilaian kuantitatif kedutan muka menggunakan fotografi membolehkan penyelidik menilai bilangan kedutan pada kawasan tertentu pada muka. Objektif kajian ini adalah untuk menentukan purata kedutan muka di kalangan wanita dalam pelbagai peringkat umur di Kelantan.

Kaedah Seramai 168 wanita berusia 20 hingga 48 tahun terlibat dalam kajian ini secara sukarela. Subjek-subjek kajian dibahagikan secara rata kepada 3 kumpulan (remaja lanjutan, dewasa awal, pertengahan umur). Imej digital muka hadapan akan diambil menggunakan peranti yang dicipta oleh OptoBioMed (Korea). Pengiraan kedutan pada glabella, dahi dan keseluruhan muka pandangan hadapan dibuat menggunakan perisian yang disediakan.

Keputusan Kedutan muka lebih ketara pada semua kawasan muka yang dikaji bagi kumpulan pertengahan umur ($p < 0.01$). Glabella menunjukkan kawasan yang lebih terjejas berbanding dahi. Dalam setiap kumpulan umur, perhubungan antara kawasan muka yang dikaji menunjukkan korelasi positif ($p < 0.05$).

Kesimpulan Keputusan kajian ini menunjukkan pertambahan kedutan muka secara progresif, terutamanya di kalangan subjek pertengahan umur. Pembentukan kedutan muka dijangkakan berlangsung secara serentak sepertimana analisis menunjukkan korelasi positif.

Kata kunci: *kedutan muka, glabella, dahi, muka hadapan, kajian imej fotografi digital*

Abstract

Background Facial wrinkles encompasses superficial and mimetic wrinkles, and facial lines; formation of which are combined effect of intrinsic and extrinsic factors. Quantitative study of facial wrinkles using photographic methods enables researchers to measure the amount of wrinkles on specific area, and in this case the face.

The aim of this study is to determine the means of facial wrinkles in female population of different age groups in Kelantan.

Methods A total of 168 women aged 20 to 48 years old, were recruited in this study based on convenience sampling. Subjects were divided equally into 3 age groups (later adolescence, early adulthood and middle adulthood). Digital photography of subject's frontal view of face at zero degree was taken using device developed by OptoBioMed (Korea). Measurement of wrinkles at glabella, forehead and frontal face were calculated using provided software.

Results Wrinkle formation was more marked at all assessed regions in the older age group ($p < 0.01$). Glabella showed an area more affected than forehead. In each age cohort, the formation of wrinkles in glabella and forehead showed positive correlation ($p < 0.05$, Pearson Correlation Test).

Conclusion The results of this present study showed progressive increase of facial wrinkles, more significantly in the older age group. The formation of facial wrinkles was expected to progress simultaneously as shown by their positive correlation.

Key words: *Facial wrinkle, glabella, forehead, frontal face, digital photographic image analysis*

BAHAGIAN C

A CROSS-SECTIONAL STUDY TO ASSESS PATTERN IN
WRINKLE FORMATION IN DIFFERENT AGE GROUPS IN
FEMALE POPULATION IN KELANTAN

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Dr. Arman Zaharil Mat Saad: Supervisor

1. INTRODUCTION

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1.1 Literature Review

1.1.1 Facial Aging Process

The human face bears many information especially in the aspects of emotional expression, inter-personal communications as well as individual identification [1]. Despite the face being relatively small part of the body, it is usually the first feature identifiable of an individual where identification is concerned [2].

One very pertinent issue that somehow gets more attention among the general population is the nascent development of facial wrinkles [3,4]. In the United States of America a total of 12.4 billion US dollars was spent in the year 2014 for surgical and non-surgical cosmetic procedures [5]. It reflects on the emerging interest and necessity of aesthetics among the general population besides the receding stigma on engaging to cosmetic procedures. In Malaysia, the blooming number of aesthetics clinics in the past decade signifies similar pace of 'beauty consciousness' among our local population.

Wrinkles can be defined as “an extension of the skin perpendicular to the axis of the wrinkle which leaves a marked line that represents the bottom of the wrinkle” [6].

A more dramatic, or genuinely more elaborated manner to look at wrinkle is by taking into account the concept of *skin aging*. Aging, in general, encompasses the habitual “wear-and-tear” effect that is affecting the normal bodily mechanical and biological functions [2]. Emre in his 2013 article on aging face laid down the perpetual aetiology

of skin aging i.e. eventual loss of collagen and elastin that subsequently creating sagging as well as relative excess of skin [2].

Aging process is inevitable. Aging of skin is possibly the most obvious and prominent, particularly of the face, since it is purportedly exposed at all times. The face also serves as the most recognizable part of the body. With the advent of latest technology and advancement in facial rejuvenation, the human population became more aware of the significance of facial wrinkle, hence the raise the urge of 'looking younger' [2]. Aging in human skin comprises of 2 components namely intrinsic (or chronological) aging and extrinsic aging, more commonly referred as photaging. Intrinsic aging process is mainly due to naturally occurring biological processes leading to appearance of thin, pale and finely wrinkled skin. On the other hand, extrinsic aging is more crudely wrinkled and contains blemishes caused by abnormal pigmentation occurs as a result of prolonged ultraviolet exposure to the skin [2,7,8].

Contrary to the saying 'Beauty is skin deep,' the tenacity of facial skin is deeper than the integumentary system per se. Mechanically, the aging of human face is the summation of both superficial textural wrinkling, as well as changes in the underlying structural component [9].

1.1.2 Superficial Textural Wrinkling

It is important to delineate the difference between the existing terms used in reference to facial wrinkles and its similar features as usage of such terms are in heavy reliance on the comprehensive idea of each meaning. Lemperle, et al (2001) suggested the definitions to wrinkles, mimetic wrinkles and skin folds [7].

Superficial wrinkles are those affected due to both intrinsic and extrinsic aging, that start as discrete and fine initially and become grouped and multidirectional over time. They are usually limited to epidermal creasing; hence tend to be responsive to superficial rejuvenation like dermabrasion, chemical peeling as well as laser resurfacing.

Mimetic wrinkles, or furrows, are result of repeated movement during facial expression and dermal elastosis. These are deep dermal creasing and lie perpendicular to the underlying facial muscle direction, for example, glabellar lines and the infamous crow's feet at the periorbital region. Treatments aiming at reducing the excursion of facial muscle including botulinum toxin injection and injectable filler materials may improve the mimetic wrinkles.

Skin folds – like nasolabial folds, and upper and lower eyelid folds in midface sagging and blepharoptosis, respectively – are caused by multiple factors including intrinsic aging, subcutaneous facial structural component laxity, gravity as well as genetic laxity. The treatment of skin folds spans a more complex spectrum including tightening procedures, and skeletal augmentation using implants, bone grafts etc.

Classification of Wrinkle Severity

Prior to the invention of more sophisticated device for quantitative and qualitative facial wrinkle assessment, clinical classification of wrinkles were important albeit limited in its overall evaluation. In 1996, Fitzpatrick introduced a classification specialised for use in perioral and periorbital wrinkling to compare the effect

following laser resurfacing. The drawback of his classification was it does not take into consideration on information relating to depth of wrinkles (Table 1) [7]. Glogau in 1996, proposed a different classification based on facial movement i.e. no wrinkles (Type I), wrinkles in motion (Type II), wrinkles at rest (Type III) and only wrinkles (Type IV). Its disadvantage is it covers generalized wrinkles and does not specifically mention skin folds or mimetic wrinkles.

Class	Score	Wrinkling	Degree of Elastosis
I	1-3	Fine wrinkles	Mild (fine textural changes with subtly accentuated skin lines)
II	4-6	Fine to moderate depth wrinkles, moderate number of lines	Moderate (distinct papular elastosis, individual papules with yellow translucency, dyschromia)
III	7-9	Fine to deep wrinkles, numerous lines, with or without redundant skin	Severe (multipapular and confluent elastosis, thickened yellow and pallid cutis rhomboidalis)

Table 1. Fitzpatrick's Classification of Facial Wrinkling (Perioral and Periorbital)

Modified Fitzpatrick Wrinkle Scale (MFWS), introduced in 2008 by Shoshani et al., offers a more elaborated clinical measurement of wrinkle severity. However in their literature [10], it was confined to the assessment of the nasolabial fold wrinkles.

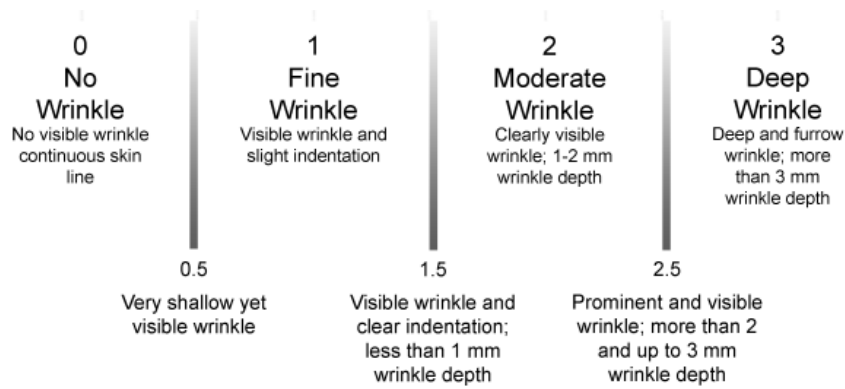


Table 2: Modified Fitzpatrick Wrinkle Scale (MFWS)

1.1.3 3-Dimensional Topographic Changes

Discussion on facial aging is not entirely complete until the contribution of facial structural component is mentioned. Coleman attributed the skeletal support and soft tissue distribution to work synergistically with skin, but are individually affected by aging process [9].

Facial skeleton aging can be result of bone loss and gradual change in relative dynamics of bone expansion. Maxillary resorption may lead to accentuation of nasolabial fold and perioral wrinkling. Tooth loss leads to marked resorption of the alveolar ridges, which makes the perioral skin structure to deflect inward.

Soft tissue profile like muscle, fascia and subcutaneous fat distribution play vital role in characterising youthful face. These tissues, especially subcutaneous fat, contribute to the 3 primary arcs that are most definitive features of youth i.e.

1. Lateral Cheek Projection

- The 'puffed' appearance runs as an unbroken convex line from lower eyelid to the cheek

2. *Arc of Jawline*

- Extending from lateral mandible to the chin

3. *Arc of Forehead*

Therefore, loss of soft tissue fullness in areas like forehead, periorbital, temporal, mandibular, glabellar and perioral regions is associated with facial aging and contributes to the obviation of facial wrinkles.

Nevertheless, fat hypertrophy and displacement alike also are associated with aging. For example, inferior displacement of orbital fat over a weakened orbital septum, which gravity also plays role in pulling of its mass, causes wider and deeper orbit and double convex deformity of the lower eyelid [11].

1.1.4 Effects of smoking and sun exposure to skin aging

Smoking and sun exposure have been long associated with skin aging. Prolonged ultraviolet exposure damaged skin down to molecular level via promotion of matrix-metalloproteinase expression, connective tissue damage, depletion of pro-collagen synthesis and increases reactive oxygen species production [3]. Smoking, besides causing serious effects to the biology of internal organs, it reduces skin perfusion hence making it impoverished of oxygen and nutrition. Smoking also increases the production of collagenase. Mimetic wrinkles may develop from squinting from exposure to cigarette smoke due to its irritating nature, or from puckering of mouth during drawing of cigarette.

1.1.5 Digital Photography in facial analysis

Usage of digital photography has improved the quantitative evaluation of skin lesion. Multiple methods have been previously practiced as non-invasive point measurement devices, however, in the advent of advance in skin care and multi-centre referrals, such methods have drawn its own drawback i.e. high risk in operator-dependent errors [12]. Hence, to reduce such errors, an objective measurement using digital colour imaging modalities have been introduced. Digital photography that is based on fluorescence and polarisation have been developed in order to allow reusable and reproducible imaging modalities as well as developing methods to analyse more objectively [12].

Digital photography, in the recent years, has developed tremendously. At such rate, the highest resolution thus far for a single digital single-lens reflex (DSLR) camera can fetch up to a whopping 50.6 megapixel (Canon EOS 5D, February 2015).

Measurement of wrinkle morphology have been developed few decades ago including conventional technique of *direct (in vivo) assessment* like confocal microscopy, which is capable of documenting photodamaged skin albeit severely operator dependent; or *indirect* method via photography or replicas [13].

1.2 Rationale of the Study

Considering the revolutionary development of facial care and nascent overgrowth of concern in youthful beauty, an objective method of measuring facial wrinkles is rendered potent and important to be developed.

In this dissertation study, where the focus of interest lies on the quantitative measurement of facial wrinkles, understanding of digital photography image analysis is crucial. Developed by OptoBioMed (Korea), the device operates with a digital single-lens reflect camera (Canon EOS Digital Rebel XT) and captures facial images in a light-restricted chamber (Figure 1). It allows three types of image illumination based on the external setting of the device. They are:

- Cross Polarisation Image (CPI)
 - This illumination gives objective analysis of deeper structure of skin including erythema index as well as melanin index
- Parallel Polarisation Image (PPI)
 - This illumination provides information of surface skin texture like wrinkles
- Ultraviolet-A Excited Fluorescent Colour Image (UV)
 - This fluorescent image provides information on sebum-related parameters e.g. condition of pores and degree of skin moisture.

The device is attached to a desktop computer containing software processor for the image obtained. Raw information on images captured and patient's data can be safely

documented and saved in the computer hard-disc.

Technically, polarised light photography yield images by applying 2 layers of linear polarisers; one in front of the camera lens, and the other at the light source (i.e. flash). Cross polarisation places both the linear polarizers at right angle, whilst parallel polarization – as the name suggests – at 0 degree. It eradicates external light source, for instance from natural sunlight, by confining object in a dark space.



Figure 1 Digital Photographic Image System, Dermavision. The inner aspect of the device (*left*). A built-in camera (Canon EOS Digital Rebel XT) is located at the back of the cuboid device with its lens projecting to the chamber. Polarising filters are located at the flash source and in front of the lens. Four ultra-violet light emitting bulbs are located at the sides of the device chamber. Forehead and chin rests are adjustable to enable fixation of head perpendicular or oblique to the lens. The external appearance of the device (*right*). A black-coloured draped is placed on top of the device to eliminate external source of light during the imaging process. The knob on top changes the mode of illumination desired.

To the interest of this study, Parallel Polarisation Image (PPI) will be emphasised in the discussion. Based on optic physics understanding, Bae Y described the mechanism of PPI by the means of enhancing skin textural features via application of spatial low-frequency pass filter into the final image [12], with the equation of:

$$I(x,y) = I_B(x,y) - B(x,y)$$

with:

$I(x,y)$: Morphologically enhanced image

$I_B(x,y)$: Original Image

$B(x,y)$: Blurred image (obtained from processing with spatial low-frequency pass filter)

Such understanding of light manipulation in extracting morphological features of facial skin allows us in the measurement of facial wrinkle. The software that is installed to the device is able to calculate percentage of wrinkle on the face as a whole or according to the region of interest (ROI) on the face.

Therefore, the objective of this study is to measure the amount of facial wrinkles in the female population objectively using the OptoBioMed's Dermavision camera and software.

2. STUDY PROTOCOL

(Documents submitted for Ethical Committee Review)

DISSERTATION PROPOSAL

A Cross-Sectional Study to Assess Pattern in Wrinkle Formation in Different Age Groups in Female Population in Kelantan

Student : Dr Zosimo Ken L. Jimeno IV

Matric No : PUM 0108/14

Programme: Master of Surgery (Plastic)

Co-investigator: 1) Dr Arman Zaharil Mat Saad

2.1 STUDY PROTOCOL

Summary of Research Proposal

The human face is the most identifiable and recognisable part of the body. Formation of facial wrinkles is inevitable given the inadvertent progress of skin aging and the perpetual environmental and bodily factors.

The study of facial wrinkles have seen tremendous effort from multiple facet of medical and non-medical camaraderie for the enhancement of knowledge and technology in this area to serve the public with the increasing awareness of aesthetic medicine.

We present this thesis proposal to measure objectively the percentage of facial wrinkle in a single population i.e. the women of Kelantan, by using digital photographic image analysis with the main objective to assess the formation of wrinkles based on the population age.

This thesis project aims to contribute to the society by becoming a benchmark for reference value of facial wrinkle percentage in a selected population.

2.1.1 Introduction

Facial Aging Process

The human face bears many information especially in the aspects of emotional expression, inter-personal communications as well as individual identification [1]. Despite the face being relatively small part of the body, it is usually the first feature identifiable of an individual where identification is concerned [2].

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Intrinsic aging process is mainly due to naturally occurring biological processes leading to appearance of thin, pale and finely wrinkled skin. On the other hand, extrinsic aging is more crudely wrinkled and contains blemishes caused by abnormal pigmentation occurs as a result of prolonged ultraviolet exposure to the skin [2,7,8].

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It is important to delineate the difference between the existing terms used in reference to facial wrinkles and its similar features as usage of such terms are in heavy reliance on the comprehensive idea of each meaning. Lemperle, et al (2001) suggested the definitions to wrinkles, mimetic wrinkles and skin folds [7].

Superficial wrinkles are those affected due to both intrinsic and extrinsic aging, that start as discrete and fine initially and become grouped and multidirectional over time. They are usually limited to epidermal creasing; hence tend to be responsive to superficial rejuvenation like dermabrasion, chemical peeling as well as laser resurfacing.

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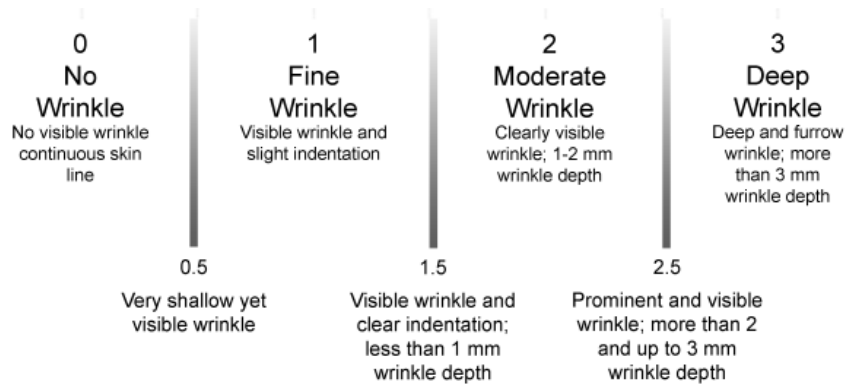


Table 4: Modified Fitzpatrick Wrinkle Scale (MFWS)

3-Dimensional Topographic Changes

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Facial skeleton aging can be result of bone loss and gradual change in relative dynamics of bone expansion. Maxillary resorption may lead to accentuation of nasolabial fold and perioral wrinkling. Tooth loss leads to marked resorption of the alveolar ridges, which makes the perioral skin structure to deflect inward. Soft tissue profile like muscle, fascia and subcutaneous fat distribution play vital role in characterising youthful face. These tissues, especially subcutaneous fat, contribute to the 3 primary arcs that are most definitive features of youth i.e.

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- The ‘puffed’ appearance runs as an unbroken convex line from lower eyelid to the cheek

2. *Arc of Jawline*

- Extending from lateral mandible to the chin

3. *Arc of Forehead*

Therefore, loss of soft tissue fullness in areas like forehead, periorbital, temporal, mandibular, glabellar and perioral regions is associated with facial aging and contributes to the obviation of facial wrinkles.

Nevertheless, fat hypertrophy and displacement alike also are associated with aging. For example, inferior displacement of orbital fat over a weakened orbital septum, which gravity also plays role in pulling of its mass, causes wider and deeper orbit and double convex deformity of the lower eyelid [11].

Effects of smoking and sun exposure to skin aging

Smoking and sun exposure have been long associated with skin aging. Prolonged ultraviolet exposure damaged skin down to molecular level via promotion of matrix-metalloproteinase expression, connective tissue damage, depletion of pro-collagen synthesis and increases reactive oxygen species production [3]. Smoking, besides causing serious effects to the biology of internal organs, it reduces skin perfusion hence making it impoverished of oxygen and nutrition. Smoking also increases the production of collagenase. Mimetic wrinkles may develop from squinting from exposure to cigarette smoke due to its irritating nature, or from puckering of mouth during drawing of cigarette.

Digital Photography in facial analysis

Usage of digital photography has improved the quantitative evaluation of skin lesion. Multiple methods have been previously practiced as non-invasive point measurement devices, however, in the advent of advance in skin care and multi-centre referrals, such methods have drawn its own drawback i.e. high risk in operator-dependent errors [12]. Hence, to reduce such errors, an objective measurement using digital colour imaging modalities have been introduced. Digital photography that is based on fluorescence and polarisation have been developed in order to allow reusable and reproducible imaging modalities as well as developing methods to analyse more objectively [12].

Digital photography, in the recent years, has developed tremendously. At such rate, the highest resolution thus far for a single digital single-lens reflex (DSLR) camera can fetch up to a whopping 50.6 megapixel (Canon EOS 5D, February 2015).

Measurement of wrinkle morphology have been developed few decades ago including conventional technique of *direct (in vivo) assessment* like confocal

microscopy, which is capable of documenting photodamaged skin albeit severely operator dependent; or *indirect* method via photography or replicas [13].

In this dissertation study, where the focus of interest lies on the quantitative measurement of facial wrinkles, understanding of digital photography image analysis is crucial. Developed by OptoBioMed (Korea), the device operates with a digital single-lens reflect camera (Canon EOS Digital Rebel XT) and captures facial images in a light-restricted chamber (Figure 2). It allows three types of image illumination based on the external setting of the device. They are:

- Cross Polarisation Image (CPI)
 - o This illumination gives objective analysis of deeper structure of skin including erythema index as well as melanin index
- Parallel Polarisation Image (PPI)
 - o This illumination provides information of surface skin texture like wrinkles
- Ultraviolet-A Excited Fluorescent Colour Image (UV)
 - o This fluorescent image provides information on sebum-related parameters e.g. condition of pores and degree of skin moisture.

The device is attached to a desktop computer containing software processor for the image obtained. Raw information on images captured and patient's data can be safely documented and saved in the computer hard-disc.

Technically, polarised light photography yield images by applying 2 layers of linear polarisers; one in front of the camera lens, and the other at the light source (i.e. flash). Cross polarisation places both the linear polarizers at right angle, whilst parallel polarization – as the name suggests – at 0 degree. It eradicates external light source, for instance from natural sunlight, by confining object in a dark space.



Figure 2 Digital Photographic Image System, Dermavision. The inner aspect of the device (*left*). A built-in camera (Canon EOS Digital Rebel XT) is located at the back of the cuboid device with its lens projecting to the chamber. Polarising filters are located at the flash source and in front of the lens. Four ultra-violet light emitting bulbs are located at the sides of the device chamber. Forehead and chin rests are adjustable to enable fixation of head perpendicular or oblique to the lens. The external appearance of the device (*right*). A black-coloured draped is placed on top of the device to eliminate external source of light during the imaging process. The knob on top changes the mode of illumination desired.

To the interest of this study, Parallel Polarisation Image (PPI) will be emphasised in the discussion. Based on optic physics understanding, Bae Y described the mechanism of PPI by the means of enhancing skin textural features via application of spatial low-frequency pass filter into the final image [12], with the equation of:

$$I(x,y) = I_B(x,y) - B(x,y)$$

with:

$I(x,y)$: Morphologically enhanced image

$I_B(x,y)$: Original Image

$B(x,y)$: Blurred image (obtained from processing with spatial low-frequency pass filter)

Such understanding of light manipulation in extracting morphological features of facial skin allows us in the measurement of facial wrinkle. The software that is installed to the device is able to calculate percentage of wrinkle on the face as a whole or according to the region of interest (ROI) on the face.

2.1.2 Objectives

General objective

To assess the percentage of facial wrinkles in female population of different age groups in Kelantan.

Specific objectives

- a. To assess the percentage of wrinkles on the face as a whole, and specifically at the glabella and forehead in different age groups among female population in Kelantan.
- b. To evaluate the difference in mean percentage of facial wrinkle between glabella, forehead and frontal face within each age group.

Hypothesis

Hypothesis: There is different value (in percentage) in wrinkles at different areas of the face at different age groups

Null Hypothesis: There is no different value (in percentage) in wrinkles at different areas of the face at different age groups

2.1.3 Methodology

Study population and sample size calculation

This is a quantitative study to determine the percentage of facial wrinkle in 168 women in Kelantan gathered through an advertisement for facial wrinkle study.

Inclusion criteria:

1. Lives in Kelantan for at least 1 year
2. Female adult, in their reproductive age, aged more than 18 years old, and below 49 years old [14]
3. Consented for involvement in this study

Exclusion criteria

1. Previous facial invasive or semi-invasive procedures, including post-facial trauma surgery and any sort of laser or surgical facial rejuvenation, done before.
2. Established chronic dermatological problems e.g. psoriasis, ichthyosis
3. Established advanced kidney disease

Demographic information of patient will be collected including (Appendix1)

1. Registration number or identification number
2. Age and Sex
3. Level of Education, Occupation and Income
4. Phone number
5. BMI with height and weight
6. Ethnicity (including parents' and grandparents')

Other relevant information will be gathered based on the standard proforma for laser treatment in Reconstructive Sciences Unit, HUSM.

1. Established medical and skin illness
2. Cosmetic History (Makeup application, facial cleansing frequency, cosmetic procedures, sun-protection cream usage)
3. Dietary history (including water and caffeinated beverage consumption and vitamin supplementation)
4. Physical activities history
5. Smoking and alcohol consumption history
6. Estimated sun exposure per week [15]
7. Gynaecological history
8. Presence of psychological stress

Sample size calculation

With the absence of previous studies that objectively measure percentage of facial wrinkles in the face, sample size calculation of this study will be based on 2 means following a small pilot study conducted among consented 10 adult subjects in the same study cohort. With compliance of the study methodology observed as proposed in item 2.1.3, the percentage of wrinkles at the mentioned ROI of the

subjects were measured using Dermavision software to obtain 3 parallel polarisation images.

Using an online software www.openepi.com, sample size for 2 means is measurable using standard deviation from the pilot study.

Following table denotes non-variable factors with its guesstimate ratio, and detectable difference based on the investigators observation:

Factors	Ratio	Standard Deviation	Detectable Difference	Sample Size Calculated	Adjustment of sample size
1) Smoking Exposure					
Smoker (Primary and Secondary)	1	2.7	1.5% of wrinkles	32	Total: 160 Estimated 10% dropout: 16 Grand Total: 176
Non Smoker	4			128	
2) Race/Ethnicity <i>(95% of Kelantanese population consists of the Malay ethnicity. For simplicity in calculation of sample size, ethnic groups are divided into Malays and Non-Malays)</i>					
Malay	1.5	2.7	1.5% of wrinkles	65	Total: 108 Estimated 10% dropout: 11 Grand Total: 119
Non Malay	1			43	
3) Age Groups <i>(Subjects will be segregated according to 3 age groups i.e. - 18 – 24 years old - 25 – 34 years old - 35 – 49 years old based on Newman & Newman’s Development through Life. Considering the distribution will be equal in each group, a ratio of 1:1 will be taken)</i>	G1 – 1 G2 – 1 G3 – 1	2.7	1.5% of wrinkles	51	Total 153 Estimated 10% dropout: 20 Grand Total 168
				51	
				51	
				51	

Therefore, from the calculation using the means from pilot study, the final grand total of sample size will be 168 subjects.