TOTAL TESTOSTERONE AND ASYMMETRIC DIMETHYLARGININE: ASSOCIATION OF DEMOGRAPHIC, CLINICAL AND LIFESTYLE FACTORS WITH THE SEVERITY OF ERECTILE DYSFUNCTION

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ABSTRACT

Erectile dysfunction (ED) is a highly prevalent disorder reported in men worldwide. ED is commonly associated with aging as well as with several other factors including demographic, clinical and lifestyle factors. However, scarce studies were reported on the combined association of demographic, clinical and lifestyle factors with worsening of severity of ED. Besides, few biomarkers too were proved to be significantly associated with ED and it is believed that demographic, clinical and lifestyle factors may have been one of the reasons which influence changes of biomarker levels in ED patients. However, these factors have not been studied extensively. Hence, this study was conducted to investigate the association of demographic, clinical and lifestyle factors with severity of ED and biomarker levels, namely total testosterone (TT) and asymmetric dimethylarginine (ADMA), and build the predictor models for severity of ED and related biomarkers.

This cross sectional study involved 276 patients. Data were recruited from patients attending ED and medical clinics in University Malaya Medical Centre, a tertiary teaching hospital in Malaysia. The demographic, clinical, lifestyle factors and severity of ED were assessed using questionnaire included with the International Index of Erectile Function (IIEF-5). Meanwhile, TT and ADMA levels were determined using the enzyme-linked immunosorbant assay (ELISA). Binary logistic regression test was used to demonstrate the predictors of severity of ED, TT and ADMA levels.

Significant predictors for worsening of severity of ED are *[presented as adjusted odds ratio (95% confidence interval), p value]* self-employed [10.55 (0.43 – 257.06), p=0.004], pensioner [8.07 (0.19 – 352.45), p=0.026], non-government employee [1.16 (0.05 – 26.26), p=0.04] and TT [0.41 (0.25 – 0.69), p=0.001]. Nevertheless, demographic and lifestyle factors were found to be the significant predictors for both TT and ADMA levels. Pensioner [0.08 (0.01 – 0.87), p=0.038] and unemployed [0.04

(0.01 –0.42), p=0.007], are the predictors which may predict the changes of TT levels. On the other hand, academic qualification (secondary) [4.51 (0.48 – 42.83), p=0.014] and intensity of physical activities (< 1 hour/day) [2.61 (0.65 – 10.48), p=0.008] were the predictors which more likely influence the changes of ADMA levels in ED patients.

In conclusion, demographic and lifestyle factors predominate clinical factors in influencing TT and ADMA levels. Demographic and clinical factors predict the worsening of severity of ED in Malaysian ED population. Identification of these predictors influencing severity of ED, TT and ADMA may help to upgrade our knowledge on risk factors of ED. Thus, these predictive models may serve as new indicators in providing primarily guidance to physicians and ensuring ED can be managed and treated more effectively.

ABSTRAK

Disfungsi erektil (ED) adalah gangguan yang sangat lazim dilaporkan di kalangan lelaki di seluruh dunia. ED selalunya dikaitkan dengan penuaan dan juga dengan beberapa faktor lain termasuk faktor demografi, klinikal dan gaya hidup. Walau bagaimanapun, kajian yang terhad dilaporkan untuk mengait factor-faktor demografi, klinikal dan gaya hidup dengan tahap keterukan ED. Selain itu, beberapa penanda biologi (Biomarker) juga telah dibukti secara signifikan dengan ED. Adalah dipercayai bahawa factor-faktor demografi, klinikal dan gaya hidup juga diramalkan sebagai salah satu faktor yang boleh mempengaruhi perubahan dalam tahap penanda biologi pada pesakit ED. Walau bagaimanapun, faktor-faktor ini belum dikaji secara meluas. Oleh yang demikian, kajian ini dijalankan untuk menilai dan mengaitkan faktor-faktor demografi, klinikal, dan gaya hidup dengan penanda biologi; Total Testosterone (TT) dan Asymmetric Dimethylarginine (ADMA) dan tahap keterukan ED serta membina model peramal bagi tahap keterukan ED dan penanda biologi.

Kajian keratan rentas ini melibatkan 276 pesakit dengan 138 kes (pesakit ED). Data telah dikumpul dari pesakit-pesakit yang menghadiri klinik ED dan klinik perubatan di Pusat Perubatan Universiti Malaya. Faktor demografi, klinikal, gaya hidup dan tahap keterukan ED dinilai dengan menggunakan soal selidik termasuk “International Index of Erectile Function” (IIEF-5). Sementara itu, tahap TT dan ADMA ditentukan dengan menggunakan Enzyme-linked immunosorbant assay (ELISA). Kaedah binary logistik regresi telah digunakan untuk menentukan peramal bagi tahap keterukan ED, TT dan ADMA.

Faktor-faktor seperti *[Dibentangkan sebagai odds ratio (95% confidence interval)]* bekerja sendiri [10.55 (0.43 – 257.06), p=0.004], pesara [8.07 (0.19 – 352.45), p=0.026], pekerja swasta [1.16 (0.05 – 26.26), p=0.04] dan Total Testosterone (TT) [0.41 (0.25 – 0.69), p=0.001] didapati menjadi peramal yang signifikan untuk tahap

keterukan ED. Manakala, faktor-faktor demografi dan gaya hidup didapati menjadi peramal yang penting bagi kedua-dua TT dan ADMA. Pesara [0.08 (0.01 – 0.87), p=0.038] dan penganggur [0.04 (0.01 –0.42), p=0.007] didapati meramalkan perubahan dalam tahap TT manakala kelayakan akademik (menegah) [4.51 (0.48 – 42.83), p=0.014] dan intensiti aktiviti fizikal (< 1 jam/hari) [2.61 (0.65 – 10.48), p=0.008] adalah peramal bagi perubahan dalam tahap ADMA pada pesakit ED.

Kesimpulannya, faktor-faktor demografi dan gaya hidup menpengaruhi TT dan ADMA manakala faktor – factor demografi dan klinikal menguasai dalam meramalkan tahap keterukan ED, pada pesakit ED di Malaysia. Pengenalpastian peramal yang mempengaruhi tahap keterukan ED, TT dan ADMA boleh membantu untuk meningkatkan pengetahuan tentang faktor-faktor risiko ED. Oleh yang demikian, model-model ramalan ini boleh digunakan sebagai panduan oleh pakar-pakar perubatan dalam memastikan ED diurus dan dirawat dengan lebih berkesan.

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LIST OF SYMBOLS AND ABBREVIATIONS

ADMA	Asymmetric Dimethylarginine
AGE	Advanced Glycation End Product
BPH	Benign Prostatic Hyperplasia
CAD	Coronary Artery Diseases
cGMP	cyclic Guanosine Monophosphate
CVD	Cardiovascular Diseases
DBP	Diastolic Blood Pressure
DDDH	Dimethylarginine Dimethylamino-hydrolase
ED	Erectile Dysfunction
ELISA	Enzyme-linked Immunosorbant Assay
eNOS	endothelial Nitric Oxide Synthase
FDA	Food and Drug Administration
HDL	High-density Lipoprotein
IIEF	International Index of Erectile Function
LDL	Low-density Lipoprotein
LUTS	Lower Urinary Tract Symptoms
NIH	National Institutes of Health
nNOS	neuronal Nitric Oxide Synthase
NO	Nitric Oxide
OR	Odds Ratio
PDE-5	Phosphodiesterase type 5
PDE-6	Phosphodiesterase type 6
PIS	Patient Information Sheet
PO2	Partial Pressure of Oxygen

RN	Reference Number
ROC	Receiver-Operating Characteristic
ROS	Reactive Oxygen Species
S.D	Standard Deviation
SBP	Systolic Blood Pressure
SPSS	Statistical Package for Social Sciences
T2DM	Type 2 Diabetes Mellitus
TMB	Tetramethylbenzidine
TT	Total Testosterone
UMMC	University Malaya Medical Centre

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CHAPTER 1: INTRODUCTION

According to National Institutes of Health (NIH) consensus panel, erectile dysfunction (ED) is defined as the inability of a man to obtain and/or maintain a penile erection that is sufficient for satisfactory sexual performance ("NIH Consensus Conference. Impotence. NIH Consensus Development Panel on Impotence," 1993; Shamloul & Ghanem, 2013). A recent study by Rahman showed that 69.5 % of Malaysians men with aged 40 to 79 years old reported had ED (Ab Rahman, Al-Sadat, & Yun Low, 2011). Since it is not life threatening, men give less attention and left it untreated. Currently more than 150 million men worldwide are affected by this sexual disorder and this number could rise to 320 million by 2025 (Meller, Stilp, Walker, & Mena-Hurtado, 2013; Nunes, Labazi, & Webb, 2012). Erectile dysfunction which was previously known as impotence is common among men aged 40 and above (Pastuszak, 2014). Few epidemiology studies reported that the prevalence of erectile dysfunction varies according to age range, countries and ethnic groups. In Malaysia, Rahman et al (2011) and Fadzil et al (2014) reported 69.5% and 62% of Malaysian aged 40 years and above have ED respectively and the incidence varies among the three main ethnic groups living in this country (Ab Rahman et al., 2011; Fadzil et al., 2014).

ED was considered as organic and psychogenic origin. Increasing knowledge on erectile function postulates that demographic, clinical and lifestyle factors could play important roles in causing severe ED in an individual. However, these factors have not been well studied particularly among Malaysian ED patients. The association of demographic factors with severity of ED can be seen with ethnicity (Momtaz, Hamid, Ibrahim, & Akahbar, 2014; K. C. Tan, Shiu, Wong, & Tam, 2011) and current marital status (Oyekanmi, Adelufosi, Abayomi, & Adebowale, 2012). Besides, the socioeconomic states such as occupation or employment status of an individual

(AlMogbel, 2014), academic qualification (Momtaz et al., 2014) and household income (Martin et al., 2014) have been found to have indirect association with severity of ED (Ab Rahman et al., 2011; Safarinejad & Hosseini, 2004). Apart from these, clinical factors such as biomarkers and type of comorbidities have been hypothesized to be strongly associated with severity of ED. Evidences signified that total testosterone (TT) and asymmetric dimethylarginine (ADMA) were found to be the most prominent biomarkers associated with severity of ED. Studies have also shown that decreased levels of TT (Brooke et al., 2014) and increased ADMA levels (Aktoz et al., 2010) play a part in the expression of nitric oxide (NO) synthesis substantially leading to progression of severe ED. There are many convincing studies of evidences that comorbidities such as type 2 diabetes mellitus (T2DM) (Tamler, 2009), coronary artery disease (CAD) (Grossmann, Gianatti, & Zajac, 2010), hyperlipidaemia (Musicki et al., 2010; Nikoobakht, Nasseh, & Pourkasmaee, 2005), hypertension (Nunes et al., 2012) and lower urinary tract symptoms (LUTS)/ benign prostatic hyperplasia (BPH) (Dutkiewicz, Skawiński, Duda, & Duda, 2012) as risk factors of ED and have been strongly associated with severity of ED. Besides that, lack of physical activity (Kałka et al., 2013) has been well documented and proven to be associated with severity of ED (La Vignera, Condorelli, Balercia, Vicari, & Calogero, 2013). In addition, studies have also shown that active and healthy lifestyles without smoking (Cao et al., 2013) and alcohol (Lee, Ho, Yip, Fan, & Lam, 2010) significantly delay the progression of severe ED.

A biological marker or biomarker is defined as biochemical alterations that are measurable in human cells or fluids and can be used as an indicator for a biological process (Micheel, Nass, & Omenn, 2012). Biomarkers are used in clinical settings to determine the status of a disease and also as an indicator of treatment response (Strimbu & Tavel, 2010). Amongst biomarkers that have been prominently associated with

erectile dysfunction are asymmetric dimethylarginine (ADMA), nitric oxide (NO), advanced glycation end product (AGE), total testosterone (TT), oxytocin, interleukin 6, sphingosine-1-phosphate and more. Out of these, TT and ADMA which have an essential role in mechanism of erection were selected for this study. TT activates and increases the synthesis of cyclic guanosine monophosphate (cGMP) by stimulating endothelial nitric oxide synthase (eNOS) synthase and increasing the expression of NO. NO is a crucial enzymes for erection. In addition, TT helps in the vasodilation of the penile arterioles around the corpora cavernosa and allows more blood inflow to maintain an erection (Isidori et al., 2014). Meanwhile, the role of ADMA as an endogenous inhibitor of NO synthase decreases the bioavailability of NO, which eventually inhibits the relaxation of smooth muscle in penile tissues and leads to ED (Javaroni & Neves, 2012). Recent studies show that elevated ADMA level in serum is strongly associated with high prevalence of ED (Aktoz et al., 2010), nevertheless ED is less prevalent in patients with high serum concentration of TT (Brooke et al., 2014). Studies have been conducted on association of demographic, clinical and lifestyle factors with biomarker levels (TT and ADMA), however the predictors influencing these biomarker levels in ED patients have not yet been identified, particularly in the Malaysian population.

There is scarcity of studies on severity of ED and biomarkers level in the Malaysian ED population. Most of the available studies on association of demographic, clinical and lifestyle factors with severity of ED and biomarkers cannot be generalized to the Malaysian population. Besides, the demographic, clinical and lifestyle factors have been studied as individual factors and linked to severity of ED. Nevertheless, to the best knowledge of the authors, no Malaysian studies have investigated the impact of combination of these factors with severity of ED and biomarkers. It is essential to study these factors and their associations in order to identify the risk factors which influence

the severity of ED and biomarker levels. Therefore, the present study was designed to assess the association of demographic, clinical and lifestyle factors with severity of ED and biomarkers in Malaysian ED patients. This study also aimed to determine the predominant predictors that influenced the severity of ED and biomarkers level in Malaysian ED patients.

1.1 Aims and Objective

1.1.1 General Objective

To evaluate the association of demographic, clinical and lifestyle factors with severity of ED and biomarkers in patients with erectile dysfunction.

1.1.2 Aims of the Study

1. To analyse the association of demographic, clinical and lifestyle factors with severity of ED in patients with erectile dysfunction.

2. To analyse the association of demographic, clinical and lifestyle factors with biomarkers in patients with erectile dysfunction.

3. To build the predictor models for association of demographic, clinical and lifestyle factors with severity of ED in patients with erectile dysfunction.

4. To build the predictor models for association of demographic, clinical and lifestyle factors with biomarkers in patients with erectile dysfunction.

1.1.3 Hypotheses

Ha: Severity of ED and biomarkers levels (TT & ADMA) are associated with demographic, clinical and lifestyle factors in patients with erectile dysfunction

Ho: Severity of ED and biomarkers levels (TT & ADMA) are not associated with demographic, clinical and lifestyle factors in patients with erectile dysfunction

CHAPTER 2: LITERATURE REVIEW

2.1 Prevalence of Erectile Dysfunction

Erectile dysfunction is one of the most common sexual problems that mainly affect men above 40 years (Pastuszak, 2014). Few epidemiological studies on erectile dysfunction reported that the prevalence of erectile dysfunction varies with age. Men in their 40's have a prevalence of ED less than 10% as compared to older men with prevalence ranging from 50 – 100% (Shamloul & Ghanem, 2013). Besides that, prevalence of ED varies according to countries of residence and ethnic groups. The percentage of ED prevalence reported in the United States, China, and India were 33.7% (Shaeer & Shaeer, 2012), 28.4% (Bai et al., 2004) and 77.3% (Goyal, Singh, & Ahuja, 2013), respectively. Meanwhile, the prevalence in our neighbouring countries like Singapore (Li, Garcia, & Rosen, 2005), Thailand (Permpongkosol et al., 2008) and Indonesia (Nicolosi, Glasser, Kim, Marumo, & Laumann, 2005) were 53.0%, 42.1 and 11.0%, respectively which were much lower than the prevalence in our country. In Malaysia, the prevalence of ED was 69.5% as reported in a cross sectional study carried out on 1331 men aged 40 and above (Ab Rahman et al., 2011). Apart from this, another study stated that 62% of hypertensive men have some form of ED and the prevalence differs among the various ethnic groups living in this country (Fadzil et al., 2014)

2.2 Mechanism of Penile Erection

Penile erectile tissues, especially the cavernous smooth muscles play an important part in the erectile process. The smooth muscles in corpora cavernosa are generally contracted during the flaccid state and only allow a small amount of blood flow to arteries around penile area. Penile erection begins when there is sexual stimulation. This stimulation generates electrical impulses along the nerves to the penis and triggers the release of nitric oxide (NO) from the nerve fibres in the penile cavernous tissue and endothelial cells of the penile arteriole (Lamina, Agbanusi, & Nwacha, 2011).

NO activates the enzyme guanylyl cyclase, which increases the production of cyclic guanosine monophosphate (cGMP) in the smooth muscle cells of the corpus cavernosa. The cGMP causes the relaxation of smooth muscles in the corpora cavernosa and leads to dilation of the arterioles and arteries, which increases the blood flow and eventually fills the corpora cavernosa to expand or erect the penis (Andersson, 2011). The role of cGMP in regulating corporal smooth muscle is crucial. The cGMP stimulates phosphorylation of proteins and regulates corporal smooth muscle tone by activating the protein kinase G, and relaxing the smooth muscle in the cavernous tissue (Francis, Busch, & Corbin, 2010). The cGMP can decrease the Ca(2+) sensitivity in smooth muscle (Bazán-Perkins, 2012) and help in the relaxation of smooth muscle in corpora cavernosa.

The expansion of penis compresses the subtunical venular plexuses between the tunica albuginea and the peripheral sinusoids, which helps to reduce the venous outflow of blood from the corpora cavernosa and thereby sustain the erection (Quasie, Martey, Nyarko, Gbewonyo, & Okine, 2010). It is noted that the arterial inflow increases up to 30-fold to make a firm erection (Mortensen, Thaning, Nyberg, Saltin, & Hellsten, 2011). Furthermore, increase in partial pressure of oxygen (PO_2) to about 90 mmHg helps to gain the erection (Douglas & Schwartz, 2010). The degradation of cGMP by an enzyme called phosphodiesterase type 5 (PDE5) causes the smooth muscles of the corpora cavernosa to contract, which leads to arteriole contraction resulting in penile detumescence (Giuliano et al., 2013). The physiology of penile erection is shown in Figure 2.1.

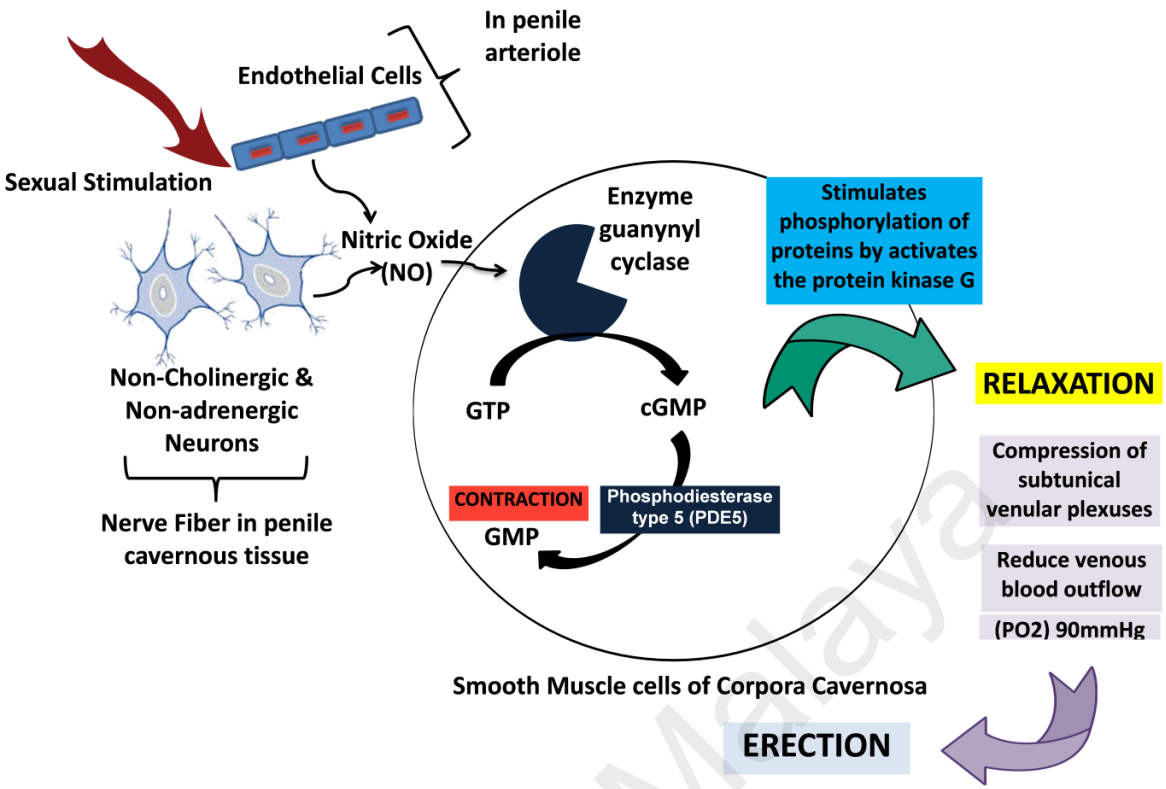

Figure 2.1: Mechanism of Penile Erection

2.3 Pathophysiology of Erectile Dysfunction

Male sexual arousal is an unpredictable and complex process which involves the brain, nerves, veins, muscles, hormones and feelings. Complications in any of these factors can result in ED. In some cases, combination of psychological and physical problems could lead to ED. ED is subdivided into two main groups; psychogenic and organic ED. Men with psychogenic ED experience constant failure to achieve or maintain an erection for a satisfactory sexual performance solely due to psychological or interpersonal factors (Rosen, 2001). However, organic ED is divided into three aetiologies, vasculogenic, neurogenic, and hormonal (Tang et al., 2014). ED was once considered as a psychogenic origin. However, findings show that most ED cases are reported due to organic causes ("NIH Consensus Conference. Impotence. NIH Consensus Development Panel on Impotence," 1993).

2.3.1 Psychogenic Erectile Dysfunction

Psychogenic ED is more prevalent among young males whose erectile function is hardly impaired by risk factors such as comorbidities or unhealthy lifestyle factors. They have less risk of having organic ED as they are usually found to be sexually active. However, they are more susceptible to impotence due to emotionally disturbed sexual life. This causes anxiety which may lead to psychogenic ED. There are few potential causes for psychogenic ED. The notable causes for psychogenic ED are performance anxiety, lack of self-confidence, stress, impaired relationship problem, psychiatric diseases and other secondary causes. Performance anxiety shows up in a man after he fails to get an erection during sexual intercourse, leading to loss of self-confidence. Performance anxieties can increase adrenalin level, which acts as a vaso-contracting agent and prevents normal erection. Besides that, stress as result of socioeconomic problem, and family or relationship issues might lead to loss of interest in sex. This can be another reason for progression of psychogenic ED.

2.3.2 Organic Erectile Dysfunction

Contradictory to psychogenic, organic ED is most prevalent in older men which are primarily caused by physical problem. Organic ED is consistent and non-selective, and men with organic impotence will not have satisfactory erection with any partner or even with the presence of certain type of stimulation. The causes of organic ED can be identified with the help of patients' physical and medical history together with laboratory result. Vascular diseases or vasculogenic is the most common cause of organic ED. Apart from that, neurogenic and endocrinologic could be the other possible causes of organic ED. Vasculogenic ED occurs due to venous leakage or an insufficient supply of blood to the arteries around the corpora cavernosa. Vascular diseases such as diabetes mellitus, hyperlipidemia or hypercholesterolemia (Giovanni & Rastrelli, et al., 2011), atherosclerosis, and hypertension (Giovanni & Rastrelli, et al., 2011; Fadzil et

al., 2014; Mittawae, El-Nashaar, Fouda, Magdy, & Shamloul, 2006; Vlachopoulos et al., 2008) are some of the well-known risk factors associated with vasculogenic ED (Graham Jackson, 2007). Arterial injuries due to these risk factors result in an inadequate blood supply into blood vessels around the penile area which causes inhibition in normal erections. Neurogenic ED is defined as impotence caused by neurological disorders or impairment due to central nervous system. The disorders may include some diseases which are often associated with ED such as Parkinson's disease, spinal cord injury, Alzheimer's disease and stroke (Siddiqui et al., 2012). Besides that, men who had undergone radical pelvic surgeries have higher chances for cavernous nerve injury which could result in neurogenic ED. Nevertheless, the advancement in current surgical methods had significantly decreased the risk of post-pelvic-surgery ED (Liu, 2010). Lastly, endocrine abnormality is cited as the main cause for endocrinologic ED. Androgen has a vital role in stimulating sexual desire and maintaining satisfactory erections in which testosterone plays an essential role in this process. Furthermore, testosterone is important in the synthesis of NO and PDE5 enzymes which are crucial for penile erection (Traish et al., 2003). Thus, testosterone deficiency or hypogonadism result in NO deficiency, leading to endocrinologic ED.

2.4 Management of Erectile Dysfunction

Nitric Oxide (NO) and cyclic guanosine monophosphate (cGMP) play key parts in the mechanism of penile erection. When there is sexual stimulation, our brain triggers the neural pathways to release NO into penis. This NO penetrates the smooth muscles around the penile area and binds to guanylyl cyclase resulting in the generation of cGMP which initiates rigid penile erection. Increasing cGMP levels in the body instigates an enzyme called PDE5 which inhibits erection by reducing the level of cGMP. Thus, the development of PDE5 inhibitors helps to inhibit the activity of PDE5. As a result, the level of cGMP increases which helps to sustain an erection (Andersson,

2011). According to Steers (2002), out of 30 million ED patients in the United States, only 4.2 million are taking medication for ED (Steers, 2002). The common PDE5 inhibitor drugs approved by food and drug administration (FDA) for the treatment of ED is sildenafil, which is commonly known as viagra, vardenafil (Levitra), and tadalafil (Cialis) (Huang & Lie, 2013). Sexual stimulation is required along with these drugs in order to attain an erection and the rigidity of penile erection is determined by the efficacy of the drugs (Hatzimouratidis et al., 2010).

2.4.1 Sildenafil (Viagra)

Sildenafil was the first oral PDE5 inhibitor approved and launched by FDA in 1998 for the treatment of ED. PDE5 inhibits accumulation of cGMP. Therefore, this sildenafil deactivates PDE5 and improves penile erection by increasing the bioavailability of cGMP level (Hakky & Jain, 2015). Sildenafil was found to be more effective in ED patients with diabetes, and prostate cancer. Besides that, it was reported to be safer for men with coronary artery disease who are not using nitrates as the concomitant drug (Doggrell, 2005). Studies have shown that sildenafil therapy for 6 months has enhanced erections by 56%, 77%, and 84% in men who took 25mg, 50mg, and 100mg of sildenafil, respectively (Goldstein et al., 2002). In addition, another study has reported that sildenafil had successfully restored erectile function by increasing the onset and duration of erection (Hadeed, Thanoon, & Al-Mukhtar, 2014).

2.4.2 Vardenafil (Levitra)

Vardenafil is the second oral medicine which was approved by FDA for the treatment of ED in 2003 (Kukreja et al., 2011). Vardenafil had the same mechanism of action as sildenafil but does not inhibit Phosphodiesterase – 6 (PDE6). PDE6 helps to alter colour perception in men, which is usually inhibited by sildenafil (Doggrell, 2005). A premarketing dose-response study was conducted to demonstrate the efficacy of vardenafil. This study confirmed that vardenafil improved erections up to 66%, 76%,

and 80% in men who took 5 mg, 10 mg, and 20 mg, respectively for 3 consecutive months. It was reported that vardenafil is effective 30 minutes after administration (Hellstrom et al., 2003).

2.4.3 Tadalafil (Cialis)

Tadalafil is the third oral medicine which was approved by FDA in 2003 to treat ED (Falk, Philip, & Schwarz, 2010). Similar to sildenafil and vardenafil, tadalafil also helps to inhibit PDE5 and uses the same mechanism of action as previous PDE5 inhibitors (Hakky & Jain, 2015). Nevertheless, tadalafil has longer duration of action and half-life of 17.5 hours (Coward & Carson, 2008). Erection was improved by 67% and 81% after patients were treated with 10 mg and 20 mg of tadalafil, respectively for 3 consecutive months (Brock et al., 2002). Headache, dyspepsia, and back pain are the most frequent side effects reported by patients who used tadalafil (Hakky & Jain, 2015). In addition, a study was carried out to determine the efficacy and treatment satisfaction in ED patients. This study concluded that 20 mg of tadalafil significantly enhances erection in men with IIEF scores less than 21 (Skoumal et al., 2004).

2.5 Classification of Severity of Erectile Dysfunction

Severity of ED is often classified as mild, mild to moderate, moderate or severe and is currently measured using the IIEF index. IIEF is the first self-administered questionnaire developed by Rosen (1997) to measure the severity of ED (Rosen et al., 1997). It contains 15 items which are used to analyse 5 major factors for a satisfactory sexual performance such as erectile function, orgasmic function, sexual desire, intercourse satisfaction and overall satisfaction (Rosen et al., 1997). Later, an abridged version of IIEF-5 was developed by Rosen (1999) containing only five items to assess and diagnose ED. Currently, this IIEF-5 questionnaire is used globally by physicians and researchers to detect and classify the severity of ED in men (Rosen, Cappelleri, Smith, Lipsky, & Pena, 1999).

2.6 Biomarkers Associated with Erectile Dysfunction

Biomarkers are used to monitor many diseases including ED. There are a number of circulating biomarkers which are robustly associated with ED; particularly total testosterone (TT), asymmetric dimethylarginine (ADMA), advanced glycation end product, interleukin 6, sphingosine-1-phosphate, oxytocin and more. Evidences revealed that TT (Brooke et al., 2014) and ADMA (Paroni et al., 2012) have been hypothesized to be the most strongly associated biomarkers with ED. The major role of TT and ADMA in regulating NO synthesis provides a strong reason on choosing these two biomarkers as potential clinical factors. A recent study conducted on 92 Turkish male patients showed a significant association of ADMA with ED, with an increase in the concentration of this biomarker being associated with a higher prevalence of ED ($r = -0.322$, $P = 0.002$). This biomarker also serves as a predictor for severe ED (OR = 14.15, 95% CI 1.10 - 181.94, $p = 0.042$) (Aktoz et al., 2010). Nevertheless, results from the Massachusetts population based cohort study revealed that ED is less prevalent in patients with high serum concentrations of TT. Increasing TT level (80% or greater)

lowered the risk of erectile dysfunction (adjusted OR 1.10 95% CI (0.59–1.98) (Kupelian et al., 2006). The important roles of these biomarkers are further discussed in sections 2.9.6 and 2.9.7.

2.7 Factors Associated with Severity of Erectile Dysfunction and Biomarkers Levels

Many factors could be significantly associated with severity of ED. Nevertheless, not many studies have been conducted with regards to the association of demographic, clinical and lifestyle factors with biomarker levels in ED patients. The factors that are hypothesized to be associated with severity of ED and biomarkers (TT and ADMA) are illustrated in Figures 2.2 and 2.3, respectively.

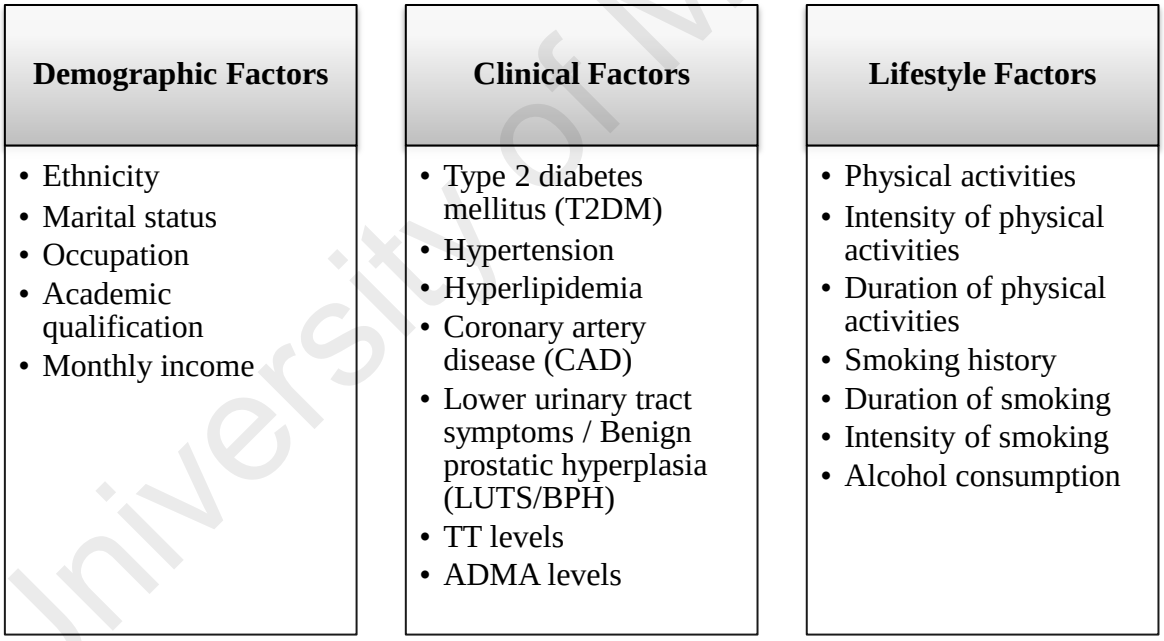

Figure 2.2: Factors Associated with Severity of ED

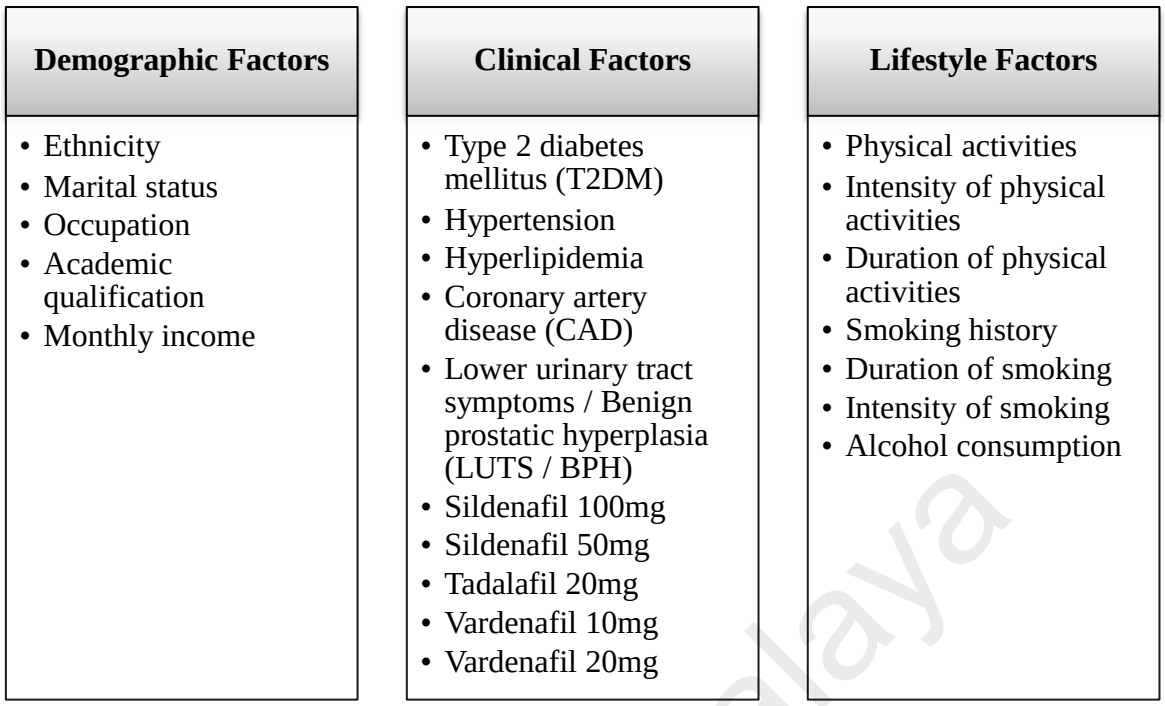

Figure 2.3: Factors Associated with Biomarkers level (TT and ADMA)

2.8 Association of Demographic Factors with Severity of Erectile Dysfunction

Demographic factors such as ethnicity, marital status, occupation or employment status, academic qualification and monthly household income were found to have indirect effects on men's erectile function.

2.8.1 Ethnicity

ED is commonly found in men of all races. Several studies found ethnic disparity to have an association with severity of ED. Findings from a cross-sectional study conducted on 351 men aged 50 years old and above at Petaling Jaya and Subang Jaya reported an insignificant association between the severity of ED and ethnicity ($\chi 2$ = 8.917, d.f. = 12, P > 0.05) (Khoo, Tan, & Low, 2008). However, a contradicting result was obtained in another study conducted on men at Subang Jaya. This multi-ethnic community-based study reported that ethnicity is significantly associated with severity of ED, with Malays and Indians being very likely to have severe ED (p = 0.001) (Tan et

al., 2007). Besides that, a recent study conducted on 1036 older married adults aged 60 years and above also revealed a significant association between ethnicity and sexual activity in the Malaysian population ($\chi 2 = 30.99$, df = 3, $p < 0.001$) (Momtaz et al., 2014). The odds ratio (OR) for having sexual activity is greater for Indians (OR = 2.12) and Malays (OR = 1.68) as compared to Chinese. A study conducted by Smith et al. (2009) involving 78, 445 California men aged 45 to 69 years revealed that Hispanic and Asian men are 1.05 and 1.1 times more likely to have moderate to severe ED as compared to white men. However, black men are less likely to have severe ED (OR: 0.54, 95% CI 0.48 - 0.61) (Smith et al., 2009). Another study that involved eight primary cares in United Kingdom reported that South Asians (OR 1.40, 95% CI 1.02–1.78) have increased odds of developing severe ED as compared to Europids (Malavige, Wijesekara, Ranasinghe, & Levy, 2015). These evidences proved that there is an association between ethnicity and severity of ED.

2.8.2 Marital Status

Limited scientific evidence on the association between the severity of ED and marital status is available. Most of these studies reported that marital status has an association with severity of ED. A cross sectional survey study conducted by Millett (2006) in 8,367 Australian men aged 16–59 years reported that erectile dysfunction is associated with marital status (Millett et al., 2006). This finding is contrary to the data obtained in a study consisting of 275 Nigerian men aged 16 and above where marital status is found to be insignificant with ED ($X^2 = 5.332$, df = 4, ($p = 0.255$) (Oyekanmi et al., 2012). The risk of having severe ED significantly differs depending on the marital status. In a study by Laumann (2008) which consists of 1,455 male blacks and Hispanics found that divorced or separated men are 1.3 times more likely to have severe ED when compared to married men (95% CI 0.7–2.4, $p<0.05$) (Laumann & Waite, 2008). Similarly, in a study involving 1,580 Western Australians men residing in and outside of metropolitan

Perth reported that the odds for having severe ED are higher among non-married (OR = 6.5; 95% CI 3.4–12.7) and separated or divorced (OR = 2.6; 95% CI 1.6–4.1) when compared to married men (Chew, Stuckey, Bremner, Earle, & Jamrozik, 2008). These studies have triggered the needs to conduct researches to study the association between marital statuses and severity of ED in order to identify the importance of the role of marital status in the onset and persistence of ED.

2.8.3 Occupation

Occupation is found to be significantly associated with severity of ED and is consistent with some studies conducted in Malaysia and globally. This statement is supported by a study conducted among 26 diabetes clinics in Israel involving 900 ED patients with diabetes mellitus whereby severity of ED is reported to be significantly increased in currently unemployed ($p < 0.0001$) and blue-collar profession ($p = 0.0044$) (Kalter-Leibovici et al., 2005) patients. The term "blue-collar" and "white-collar" are occupational classifications that differentiate workers who perform manual labour and professional jobs respectively. Similar report is obtained from a study conducted by Aytac (2000) that revealed a significant association between occupation or employment status with severity of ED. The result demonstrated that men in blue-collar professions are 1.55 times more likely to acquire severe ED when compared to men in white-collar occupation (O.R.=1.55, 95% C.I.=1.06–2.28) (Aytaç, Araujo, Johannes, Kleinman, & McKinlay, 2000). Furthermore, a study involving 312 Saudi diabetic patients with ED reported that unemployed men are more likely to develop severe ED as compared to employees, and significant association was obtained between occupation and severity of ED ($X^2 = 29.069$, df = 3, $p = 0.001$) (AlMogbel, 2014). Stress due to unemployment or work burden and financial crisis faced by unemployed men causes their ED to be untreated over a long time. This could also lead to the risk of increasing ED severity.

2.8.4 Academic Qualification

Higher academic qualification level is associated with increased self-awareness on state of health (Wyllie, 2005). A positive association is observed between severity of ED and lower level of education in a study conducted by Laumann et al. (2008) whereby the risk of having severe ED is slightly lower in "higher degree group (Master / Doctorate degree)" (OR = 1.7, 95% CI 1.0 – 3.1) than "college degree group" (OR = 0.64, 95% CI 0.44 - 0.91) as compared to education level lower than high school (Laumann & Waite, 2008). Besides that, a study conducted on Italian men also showed that the prevalence of ED is higher among men with lower education level with odds ratio for having severe ED being higher in men with secondary education (OR = 0.8, 95% CI 0.7-0.9) than tertiary education (OR = 0.7, 95% CI = 0.6-0.9) (university degree) (Mirone et al., 2004). Furthermore, a Portuguese ED study conducted between July 2004 and January 2005 in 50 primary healthcare centres reported that men with 4^{th} grade education level (OR = 1.34 95% CI 1.03–1.75) have higher odds to have severe ED as compared to men who have completed 7^{th} grade education (OR = 1.25 95% CI 0.96–1.62) (Teles et al., 2008). It can be inferred that educated men are mostly employed, highly paid and therefore have no barrier in accessing primary health care centres in order to maintain a healthy life. They also have sufficient knowledge on ED management which will eventually help them to live a quality lifestyle (Momtaz et al., 2014).

2.8.5 Monthly Income

Many studies have proved that income is one of the crucial demographic factors which are significantly associated with the severity of ED (AlMogbel, 2014; Martin et al., 2014). Furthermore, the risk of acquiring severe ED increases as household income decreases (Martin et al., 2014). A study conducted by Tan et al. (2003) involving 729 Singaporean men revealed that lower income level is categorised as an essential risk

factor for acquiring severe ED for men aged 30 and above (Tan, Hong, Png, Liew, & Wong, 2003). Similarly, a study conducted on 1,300 men aged 18 years and above acquiring primary healthcare service in Durban, KwaZulu-Natal reported that men with low monthly income (< 5000 Rands) are 2.01 times more likely to have severe ED as compared to men who earn more than 5000 Rands per month (OR = 2.01, 95% CI = 1.30 – 3.01) (Lockhat, Ross, Ramlachan, & Rangiah, 2013). In addition, a study by Momtaz et al. (2014) conducted on 633 men in Malaysia also reported a significant association between household income and severity of ED (X^2 = 24.01, df = 2, p < 0.001). This study also revealed that men with higher educational level are well-paid and had greater control over their own health and lives (Momtaz et al., 2014). ED patients who earn more tend to spend their income wisely and pay more attention on health issues. In contrast, men with lower income give first priority for their basic needs as compared to ED treatment which eventually leads to their ED being left untreated (Hackett, 2005).

2.9 Association of Clinical Factors with Severity of Erectile Dysfunction

ED often overlaps with many risk factors especially clinical factors. In spite of the fact that the origin of ED is multifactorial, comorbidity can be the primary contributing factor for ED, especially T2DM, hypertension, hyperlipidemia, CAD and LUTS/BPH. In addition, some biomarkers are found to be associated with severity of ED too.

2.9.1 Diabetes Mellitus (Type 2 Diabetes Mellitus)

Diabetes mellitus is a common chronic disease among ED patients (Tamler, 2009). Vascular and neurological diseases (vasculopathy and neuropathy) can be major risk factors of ED in diabetes patients. Decreased eNOS expression results in decreasing bioavailability of NO due to endothelial cell dysfunction and impaired vascular smooth muscle relaxation resulting from the deficiency of nitric oxide–cGMP pathway and increasing risk of ED severity in patients with diabetes (Maiorino, Bellastella, &

Esposito, 2014). Other than that, factors such as impaired vasodilatory signalling, cavernosal hypercontractility, and veno-occlusion interfere with the erection mechanism and lead to the development of severe ED in patients with diabetes (Hidalgo-Tamola & Chitaley, 2009). Many studies found similar findings and diabetes is accepted as a risk factor for worsening of ED severity. A cross-sectional study conducted by Ugwu et al. (2016) involving 160 men aged 30–70 years found the duration of diabetes mellitus as an independent predictor for severity of ED (1.14 95% CI 1.02–1.28) (Ugwu, Ezeani, Onung, Kolawole, & Ikem, 2016). It is a well-known factor that diabetes-related ED originates from vasculogenic problem due to vascular complications. The longer a person has diabetes, the worse the complication would be, and this would eventually lead to severe ED. Besides that, risk factors such as testosterone deficiency and poor glycemic control could be another risk factor for severe ED (Ugwu et al., 2016). Similar to the observation by Ugwu et al. (2016), a cohort study reported that men with diabetes mellitus have a higher risk of severe ED as compared to men without diabetes. The risk of getting severe ED is 1.3 times greater in patients with type 2 diabetes (RR 1.3, 95% CI 1.1–1.5), and three times higher in patients with type 1 diabetes mellitus (RR 3.0, 95% CI 1.5–5.9). However, men with type 2 diabetes have a higher risk of severe ED with increasing duration of diabetes with relative risk of 1.7 (RR 1.7, 95% CI 1.1–2.7) (Bacon et al., 2002). These studies explained that the duration of diagnosis could also increase the risk and severity of ED in diabetic patients.

2.9.2 Coronary Artery Disease (CAD)

ED is highly prevalent in patients with cardiovascular diseases (CVD) such as myocardial infarction, angina pectoris, congestive heart failure and especially coronary artery disease (CAD) (Grossmann et al., 2010). The strong link between ED and CAD is because both these conditions share similar underlying aetiological factors. The vascular system plays an important part in supplying adequate blood supply to the cavernosal

tissue and in maintaining erection by reducing blood outflow, which is made possible through the compression of subtunical venular plexuses (Dean & Lue, 2005). Therefore, changes or problems in the vascular system and vascular disturbance of the endothelium can increase the possibility for severe ED. In addition, endothelial dysfunction due to vascular complication interrupts NO production and prevents the relaxation and vasodilation of the vascular tissue lining the inner part of arterioles, which eventually worsens the severity of ED (Huri, Choo, Sulaiman, Mark, & Razack, 2014). A number of studies have been carried out to examine association of CAD with severity of ED. A meta-analysis of 12 prospective cohort studies involving 36,744 patients identified CAD as a significant predictor for severe ED with odds ratio of 1.46 (95% CI: 1.31-1.63) (Dong, Zhang, & Qin, 2011). Furthermore, another study by Thompson et al. (2005) revealed that out of 4,247 men with cardiovascular complications, 2,420 (57%) had reported severe ED after 5 years of CAD, and the percentage increased to 65% after 7 years (Thompson et al., 2005). Besides that, ED is considered as a significant early predictor of CAD and ED patients are advised to undergo medical tests to avoid any cardiovascular mortality (Jackson et al., 2010).

2.9.3 Hypertension

Hypertension is reported to be significantly associated with severity of ED. Both hypertension and ED have endothelial dysfunction as a common risk factor (Nunes et al., 2012). The bioavailability of NO is found to be lower in hypertension patients. An imbalanced mechanism between pro- and anti-oxidants in endothelial cells increases the reactive oxygen species (ROS) generation. Thus, the interaction between NO and ROS eventually decreases the NO availability, which leads to failure in relaxation of cavernosal tissue, causing risk of having severe ED (Nunes et al., 2012). A higher prevalence rate is observed in a study conducted in Malaysia among hypertensive patients where 62% of them have had ED (Fadzil et al., 2014). This rate increases

significantly with age, differs among various ethnic groups and is found to be higher in patients with diabetes mellitus as a concomitant disease with hypertension (Fadzil et al., 2014). Another study conducted by Giuliano showed that out of 3,906 hypertensive patients, 2,379 (61%) are reported to have ED with mean IIEF score of 12.0 ± 4.6 that falls under the ED severity category of "mild to moderate" (Giuliano, Leriche, Jaudinot, & de Gendre, 2004). Another study conducted by Zedan et al. (2010) on 658 ED patients found that hypertension is 5.4 times more likely to have increased severity of ED among Egyptian men (Zedan, Hareadei, Abd-Elsayed, & Abdel-Maguid, 2010). These studies showed that hypertension may be associated with severity of ED and studies are needed to prove it.

2.9.4 Hyperlipidemia

Hyperlipidemia is one of the main risk factors for vasculogenic ED which mostly affects the endothelial and smooth muscle cells of the penis (Vrentzos, Paraskevas, & Mikhailidis, 2007), and the peripheral nerves for penile erection (Rao, Du, & Yang, 2006). Endothelial dysfunction and the decrease in NO synthesis have become the major problem in hyperlipidemic patients. These issues lead to severe ED (Vrentzos et al., 2007). Furthermore, oxidized low-density lipoprotein (LDL) is another causative factor for the impaired relaxation response of the corpus cavernosa (Vrentzos et al., 2007). A study conducted by Rao also shown that occlusion and arterial stenosis caused by hyperlipidemia could be attributed to severity of ED (Rao et al., 2006). The association between hyperlipidemia and severity of ED has been documented in several research studies. Total cholesterol, triglyceride, and LDL are positively correlated with severity of ED whereas, an increased high-density lipoprotein HDL level is negatively correlated with the severity of ED (Nikoobakht et al., 2005; Rao et al., 2006; Yassin et al., 2015). Roumeguere reported that the prevalence of hyperlipidemia in ED patients (n = 215) is 70.6% as compared to 52% in patients without ED (n = 100). This study also

concluded that the serum HDL cholesterol level and the total cholesterol/HDL cholesterol ratio could be used as predictors of ED severity level (Roumeguere, Wespes, Carpentier, Hoffmann, & Schulman, 2003). Besides that, the occurrence of abnormal LDL is significantly higher in ED patients (n = 943) with 32.4% as compared to control group without ED (4.1%, n = 242). This result suggests that LDL is strongly associated with severity of ED. The correlation between plasma level of total cholesterol and LDL with the IIEF-5 score is demonstrated in the study by Nikoobakht, whereby for every mg/dl increase in cholesterol and LDL, there is a decrease of 0.036 and 0.035 in the IIEF-5 scores, respectively (Nikoobakht et al., 2005). In addition, significant association is reported between triglycerides (P = 0.009) and total cholesterol (P = 0.027) with severity of ED (Yassin et al., 2015). A study conducted by Cordero et al. (2010) involving 715 ED patients indicated hyperlipidemia as a predictor for acquiring severe ED (OR 1.28, 95% CI 0.81–2.01). Hyperlipidemia may induce severe ED by interrupting the synthesis of NO and provoking impairment in the relaxation of smooth muscle of corpora cavernosum. This impairment eventually results in erection problem which significantly increases severity of ED.

2.9.5 Lower Urinary Tract Symptoms/ Benign Prostatic Hyperplasia

Benign prostatic hyperplasia (BPH) and ED are totally different problems and prevalence of both disorders increases with age. However these disorders are somehow related to one another. Studies have shown that there is an association between BPH and ED. The connection between BPH and ED is complicated but men who are diagnosed with BPH will have an affected erectile function (McVary, 2005). A few studies have indicated that BPH accompanied with lower urinary tract symptoms (LUTS) are associated with increasing incidence of severe ED. There are several evidences indicating that a few medications for BPH and LUTS can cause ED. A Male Cologne Survey interview involving 4,489 men aged 30 years and above surveyed patients'

sexual life. This study found that men having LUTS due to BPH had ED and the percentage increases with age: 38%, 43%, 72%, 79% and 75% in men whose ages lie between the age ranges of 30 to 39, 40 to 49, 50 to 59, 60 to 69 and 70 to 80, respectively (Braun et al., 2000). Besides that, another study conducted among 3,230 men with LUTS and BPH from Asia, Middle East, Europe, Latin America and Russia found that they are two times more likely to have severe ED (Emberton et al., 2003). In addition, a multinational survey conducted on United States and European countries found that men aged 50-80 years old demonstrated 2 to 11 fold increased risk of having severe ED in men reported having BPH with LUTS (Rosen et al., 2003). Researchers are still ambiguous on how BPH is associated with severe ED. Mulhall (2011) reported that nerve fibres in the sympathetic nervous system transmit a signal which induces stress and its related symptoms. Thus, an increase in this signal in men with BPH-associated LUTS leads to over-activity in the sympathetic nervous system and this in turn results in severe ED (Mulhall, 2011).

2.9.6 Total Testosterone (TT)

Total testosterone (TT) plays a crucial role in the sexual function of men and several studies have investigated the relationship between TT levels with male sexual behaviours especially ED (Liao et al., 2012; Tsujimura, 2013). According to a systematic study by Mikhail, a high level of TT may not have a major impact on erectile function, but if it is below normal level, there may be major impacts (Mikhail, 2006). Study conducted on 1,776 Chinese men aged 20 to 77 years old showed that TT is significantly associated with severity of ED with an odds ratio of 1.02 after adjusting the confounding variable; age (OR: 1.02; 95% CI; 1.00–1.04) (Liao et al., 2012). This finding was consistent with the evidence from a study by Brooke et al. (2014) involving 126 ED patients with type II diabetes, which reported a significant positive correlation between IIEF-5 score and TT ($r = 0.546$, $p = 0.001$), where the severity of ED decreases

as TT level increases (Brooke et al., 2014). Parallel to this finding, a prospective study by Kang et al. (2011) involving 134 men who visited urology clinics with the complaint of ED revealed a significant positive correlation between serum TT level and IIEF scores (r=0.124, p=0.034). This study also disclosed that TT levels below the normal range significantly increased the severity of ED (Kang, Ham, Oh, Kim, & Moon, 2011).

2.9.7 Asymmetric Dimethylarginine (ADMA)

Asymmetric dimethylarginine (ADMA) is a biomarker that is mainly associated with severity of ED. Few studies has been found to support this hypothesis. A case control study participated by 92 male patients who underwent exercise electrocardiography and coronary angiography has reported that plasma concentration of ADMA is significantly higher in ED patients (0.75 ± 0.40 μmol/ml) as compared to the control group without ED (0.50 ± 0.25 μmol/ml, p = 0.021) (Aktoz et al., 2010). The study also concluded that ADMA could be a biomarker of ED and elevated ADMA level could be used as an indicator to determine the severity of ED. In this study, Aktoz reported that ADMA may be considered as a potential biomarker of CAD too. ED and CAD share the common risk factors; which are generalized vascular diseases and endothelial dysfunction (Aktoz et al., 2010). Consistent with this, a study conducted on 61 men with arteriogenic and non-arteriogenic ED patients also showed that the ADMA concentrations are higher in men with arteriogenic ED compared to patients with non-arteriogenic ED and controls ($p < 0.05$). A negative correlation is present between ADMA level and IIEF scores in patients with arteriogenic ED (Paroni et al., 2012). Thus, severity of ED worsens with increase in ADMA level. Additionally, another retrospective study by Loakeimidis *et al*. (2011) involving 104 vasculogenic ED patients without CAD history showed ADMA levels is significantly higher in ED patients compared to without ED (0.65 ± 0.13 vs 0.53 ± 0.11 μmol/l; $p < 0.001$). Besides that, ADMA levels reportedly increased in men whose ED duration was more than two years (0.69 ± 0.13 μmol/l) compared with

patients whose ED duration was less than 2 years (0.60 ± 0.11 μmol/l; p = 0.03) (Ioakeimidis et al., 2011). This study found venous leakage and insufficient penile arterial inflow as the result of increased ADMA levels that offer a mechanism which lead to severe ED (Ioakeimidis et al., 2011). ADMA is remarkably known as an endogenous inhibitor of eNOS, which decreases the synthesis of NO. Erectile function is very much dependent on NO for vasodilation of corpus cavernosum and a rigid penile erection. Elevated ADMA results in NO deficiency which in turn increases the risk of severe ED (Gareri, Castagna, Francomano, Cerminara, & De Fazio, 2014).

2.10 Association of Lifestyle Factors with Severity of Erectile Dysfunction

Clinical factors could be the major cause of severe ED in men and many medical treatments are available for curing or reverting severe ED into mild or normal. However, it is surprised to learn that lifestyle changes also can contribute a significant impact on severe ED. Whether it is poor physical exercise, heavy smoking or excessive alcohol consumption can have an effect in achieving sufficient erection and could lead to severe ED.

2.10.1 Physical Activities

Physical activity was proven to be significantly associated with severity of ED. A number of studies have proven that physical activities have positive impact on erectile function. Physical activity improves erectile function by reducing metabolic disturbances like inflammatory markers and insulin resistance. Besides that, it also helps in decreasing visceral adipose tissue, and improving vascular function. Furthermore, physical activities could recover both erectile and endothelial dysfunction and better results can be observed with balanced diet and proper medication (Vignera, Condorelli, Vicari, D'Agata, & Calogero, 2012). A cross sectional survey conducted among US men reported that ED was more prevalent in men who do not carry out physical activities (23.3 %, 95% CI, 20.0 - 27.0) as compared to men who are involved in vigorous activity

(12.6%, 95% CI, 9.8 - 16.2). Men who do not involve in physical activities are 1.94 times more likely to have severe ED (OR = 1.94, 95% CI, 1.32-2.83). This suggests that involvement in physical activities could enhance sexual function of male (Selvin, Burnett, & Platz, 2007). Besides that, this study also revealed physical inactivity as a potential risk factor of severe ED. Parallel to this finding, a similar result was obtained in a recent study participated by men with arterial ED assessing the impact of aerobic exercises on severity of ED (150 minutes per week). Significantly higher IIEF scores were observed in intervention group compared with control group after being exposed to aerobic physical activity for 3 months consecutively ($P < 0.05$) (La Vignera, Condorelli, Vicari, D'agata, & Calogero, 2011). In addition, a randomized double-blind study conducted in Nigeria involving ED patients with hypertension showed an improvement in IIEF scores after a rigorous exercise training for 8 weeks (45 – 60 minutes per day) (Lamina, Okoye, & Dagogo, 2009), supporting that lifestyle changes in term of increasing physical activity may significantly decrease severity of ED.

2.10.2 Smoking

Many investigations have reported that smoking is positively correlated with severity of ED. Current and passive smokers are at higher risk of having ED than non-smokers who are not exposed to smoke. Those exposed to smoke are 1.4 times more likely to have increased risk of having severe ED (OR = 1.4) (Shiri et al., 2005) whereas smoking cessation could improve erectile function (Kovac, Labbate, Ramasamy, Tang, & Lipshultz, 2015). Also, the intensity of cigarette smoking has a powerful association with severity of ED (Pourmand, Alidaee, Rasuli, Maleki, & Mehrsai, 2004). In a study conducted among Chinese men (n = 2,686), it is reported that heavy smokers (those who smoke more than 20 cigarettes per day) have the highest odds ratio of having severe ED as compared to non/ex-smokers (OR = 1.23, 95% CI: 1.03, 1.49, p = 0.02) (Wu et al., 2012a). A meta-analysis study conducted by Cao in 2013 involving more

than 28,000 participants showed that current smokers are 1.51 times most likely having severe ED compared with non-smokers (95% CI: 1.34-1.71) (Cao et al., 2013). In addition, smoking also may increase occurrence and severity of ED by two-fold and heavy smokers are at high risk to be impotent (Mcvary, Carrier, & Wessells, 2001; Mirone et al., 2002). Intense smoking could cause severe ED through several mechanisms. Penile NOS activity and neuronal NOS (nNOS) content gradually decreased in passive smoking rats, indicating that smoking could degenerate the bioavailability of NO, which is essential for erection (Tostes et al., 2008). Protein expression of nNOS and dimethylarginine dimethylaminohydrolase (DDAH) were down-regulated with cigarette smoke extract, which results in the accumulation of NOS inhibitors such as ADMA and monomethylarginine. These inhibitors could interrupt the NO–cGMP pathway in corpora cavernosa and modulate the penile erection (Imamura et al., 2007). At the same time, smoking causes significant damage to the vascular endothelium and peripheral nerves leading to degeneration of eNOS and increment in ROS generation, which affect NO production (CDC, 2010) and subsequently cause severe ED.

2.10.3 Alcohol consumption

Persistent alcohol consumption may exert a serious effect on sexual dysfunction and could cause severe ED; however only limited studies were reported on the role or the effect of alcohol on penile erection. In particular, a study conducted in Bangalore including patients diagnosed with alcohol dependence syndrome showed 33.3% out of 96 alcohol dependent male subjects have reported having severe ED (Arackal & Benegal, 2007). Consistent to this, study by Lee *et al.* (2010) revealed that severe ED is reported in male alcoholics as compared to non-alcoholic controls with OR value 2.27 after adjusting confounding variable age and cigarette smoking (OR = 2.27, 95% CI = 1.28 - 4.03) (Lee et al., 2010). However, contrary to this a recent study involved 810

Australian men aged 35 to 80 years old reported low-alcohol consumption as predictor of severity of ED (Martin et al., 2014). Heavy alcohol consumption would cause serious neurotoxic effects leading to reversible vagal neuropathy (Arackal & Benegal, 2007). However, chronic alcohol intake may alter gonadal hormones and cause hypogonadism, which in turn may affect neural transmission to the penile tissue (polyneuropathy) and eventually lead to severe ED (Hood & Kirby, 2004). More researches need to be conducted to find the contributing factors of alcohol consumption to ED severity.

2.11 Association of Demographic Factors with Biomarkers (TT and ADMA)

Many studies have been recorded to prove that demographic factors have been significantly associated with severity of ED. Nevertheless, the understanding on association of demographic factors with biomarker levels is debatable. Very limited studies have been explored to associate demographic factors with TT and ADMA levels especially in ED patients. The output of this association study may help to identify the role of demographic factors in influencing biomarker levels among ED patients.

2.11.1 Ethnicity

A cross sectional study conducted in Malaysia involving healthy adult men (n = 189) aged 20 to 39 years old showed an insignificant association between TT level and ethnicity ($p > 0.05$) (KokYong, ImaNirwana, IsaNaina, Hanapi, & WanZurinah, 2012). However, this study consists of Malay and Chinese populations only which do not cover all the major ethnicities in this country. Besides that, a population-based community survey conducted at 7 health centres in Manchester, UK participated by men aged 25 to 74 years old found low TT level being reported in Pakistani men (14.6 nmol/l; 95% CI 12·6–16·6 nmol/l) compared to European (18.7nmol/l; 95% CI 16·8–20·6 nmol/l) and African-Caribbean men (18.0 nmol/l; 95% 16·4–19·6 nmol/) ($F = 4.8$, $P = 0.009$)

(Heald et al., 2003). The differences in TT levels among these races are multifactorial in origin and larger studies with more subjects are needed to establish normative levels.

Scarce studies had been conducted to study the association of ADMA with ethnicity in Malaysia. Globally, a cross sectional study performed by Sydow *et al.* (2010) on adults aged 60–72 years (n = 980) reported that mean ADMA concentrations are significantly higher in whites (0.63 μmol/L) as compared to African Americans (0.60 μmol/L; P = 0.01) and mixed non-Hispanics (0.60 μmol/L; P = 0.05) (Sydow et al., 2010). However, this study was conducted on healthy older men without ED.

2.11.2 Marital Status

A study involving North American men (n = 122) was the first study conducted to examine variation in TT level among married and unmarried men (not committed or involved in any relationships) (Burnham et al., 2003). Results demonstrated that married men had 21% lower TT level as compared to unmarried men and this gives a significant association between marital status and TT level (p = 0.006) (Burnham et al., 2003). This result is consistent with another recent study conducted in North America (n = 120) which reported that salivary TT level is significantly lower in married or committed men than it is in unmarried or single men ($F(2,70) = 4.18$, p = 0.019) (Van Anders & Goldey, 2010). A similar result was obtained in a study by Hooper (2011) which reported that marital status (relationship commitment) is significantly associated with TT (P < 0.01), with increasing commitment levels (married or in relationship) predicting lower TT levels among American men (P < 0.05) (Hooper, Gangestad, Thompson, & Bryan, 2011).

To the best of our knowledge, no study has been conducted to examine the association of ADMA with marital status in Malaysia and worldwide and the results from our study will provide important information for future studies.

2.11.3 Occupation

A number of studies had been conducted on the effect of type of occupation on biomarker levels. However, there are scarce studies that have been carried out in researching the association of occupation with TT and ADMA levels. Working environment and occupation burden may affect testosterone level in men (Wicks, 2012). Studies have investigated that testosterone level may vary according to varying occupations (Alvarado, 2013; Sancini et al., 2011). A population based study involving employed men aged 16 years and above (n = 414) examined the association between serum TT levels and occupation. This study specifically analysed the association between TT levels and occupations which were exposed to high noise exposure. The occupation that experienced a high noise exposure had decreased the TT levels (95 % CI: -111.22, -5.42, $p < 0.05$) (Dzhambov, 2016). A contrasting result was obtained in a study exploring the association between TT levels and self-employment (entrepreneurial behaviour). This study which tested using two large population based on data samples from the 'Rotterdam Study' (n = 587) and the 'Study of Health in Pomerania' (n = 1697) reported no significant association between self-employment and serum TT levels respectively (95% CI: 0.018, 0.109, $p = 0.163$) (95% CI: 0.030, 0.053, $p = 0.602$) (Van der Loos et al., 2013).

No recent studies explored on the association of occupation with ADMA level. Nevertheless, similar to TT, ADMA level might be affected by the occupations which were exposed to high noise exposure.

2.11.4 Academic Qualification

Limited studies have been conducted on association on academic qualification with biomarker levels (TT and ADMA). Nevertheless, none of these studies were conducted on ED population. A cross sectional study conducted among Jordan men aged 30 to 70 years with T2DM (n = 1089) revealed TT level is significantly associated with

academic qualification (p = 0.005), and it is found to be higher among university graduates (69.4%, ≥ 3ng/ml) as compared to non-university graduates (Al Hayek et al., 2013). In addition, bioavailable testosterone too is significantly associated with education (p=0.044) whereby the risk for high bioavailable testosterone is greater in men with high education compared to those with a lower education (2.32; 95% CI: 1.49–3.64; P = .0002 vs. 0.95 95% CI: 0.64–1.40; P = .790) respectively (Carcaillon et al., 2014). Nevertheless, the association of academic qualification with ADMA is still highly debated on. Educated men are highly aware and knowledgeable of their health issue as compared to those with lower education (Momtaz et al., 2014). Apart from this, they are also willing to consult with the physician to treat their problem.

2.11.5 Monthly Income

To the best of our knowledge, no studies have been conducted to associate income with ADMA level. However, a few studies have been reported on the association of TT levels with income. A cross sectional study by Al Hayek *et al.* (2013) participated by 1,089 diabetic men identified income as a risk factor that is significantly associated with low serum TT (p = 0.005). Besides that, multivariate analysis indicated men with lower monthly income are 1.76 times more likely to have low TT level than those who with higher monthly income (OR; 1.76, 95% CI: 1.35 – 2.29, p = 0.005) (Al Hayek et al., 2013). Their lower income can be a reason to limit access to health care centre. Besides that, financial crisis may lead to poor health care and increases stress level which may eventually influence both TT and ADMA levels.

2.12 Association of Clinical Factors with Biomarkers (TT and ADMA)

Comorbidities which are associated with severity of ED have been postulated to influence biomarker levels in ED patients. A number of studies have been published on these; however studies on association of ED drugs with biomarker levels are very limited.

2.12.1 Diabetes Mellitus (Type 2 Diabetes Mellitus)

Few research studies have proved that there is an association between biomarkers and diabetes mellitus. TT is found to be decreased (Grossmann et al., 2010), and the ADMA level elevated ($p = 0.001$) (Yaşar et al., 2011) in men with type 2 diabetes mellitus (T2DM). A meta-analysis study ($n = 37$ articles) reviewing the association of TT with T2DM showed that low TT level was significantly associated with T2DM with adjusted r value = -0.568 ($p < 0.0001$) in comparison to non-diabetic patients. Besides, in comparison with the control group (without diabetes), low TT levels were recorded among diabetes patients (HR= -2.08; 95% CI: -3.57, -0.59; $p < 0.001$) and similar result was demonstrated when T2DM individuals with and without ED were analysed separately (Corona, Monami, et al., 2011). In addition, a cross sectional study participated by elderly Chinese men ($n = 492$) demonstrated that serum TT is significantly lower in diabetes mellitus patients (13.8 ± 4.7 nmol/L) as compared to control patients without diabetes (17.1 ± 6.1 nmol/L; $P < 0.01$) (Cao et al., 2011). However, Kapoor *et al*. (2007) evaluated 198 T2DM men and reported no significant difference in TT levels between ED and non-ED patients. However, the study reported severity of ED is significantly associated with TT levels in ED patients ($r = 0.32$; $p < 0.001$) (Kapoor, Clarke, Channer, & Jones, 2007).

Serum concentration of ADMA is found to be significantly elevated in both patients with diabetes and ED, nevertheless limited studies were recorded. A study carried out in Istanbul, Turkey involving 58 diabetic patients and 26 healthy volunteers (control) showed a significant difference in ADMA level between diabetes and non-diabetes patients ($P = 0.0001$). In addition, a higher ADMA level was obtained in the diabetic group (0.52 ± 0.49 ng/ml) compared to control (0.06 ± 0.06 ng/ml) (Yaşar et al., 2011). ADMA which inhibits the synthesis of NO, presents a possible mechanism for the association between diabetes and ED (Yaşar et al., 2011).

2.12.2 Coronary Artery Disease

Similar to diabetes mellitus, TT level was decreased in CVD patients especially those with CAD (Malkin et al., 2010; Rosano et al., 2007; Tirabassi et al., 2013). A cross sectional study was conducted on outpatients (n = 244) who attended clinics at Institute of Tropical Medicine in Antwerp, Belgium in which it was reported that low levels of testosterone is found in 36.7% of coronary heart diseases patients with ED (De Ryck, Van Laeken, Apers, & Colebunders, 2013). However, few of the patients were HIV infected and this probably could be a cause of low level of testosterone. Hu (2011) reported that TT levels were significantly lower in patients with CAD (n = 87), and the multiple regression analysis reported TT as a predictor for CAD (OR = 0.311, 95% CI: 0.174–0.512) (Hu et al., 2011). In parallel with this, a study conducted in Japanese men (30 to 69 years old) with coronary risk factors showed that those with low TT levels had 3.67 fold higher CAD events after adjustment of confounding factors including medication (HR = 3.61, 95% CI: 1.47–8.86) (Akishita et al., 2010). It is possible that, low TT levels increased vascular damage following CAD which could lead to increased severity of ED (Corona et al., 2010).

In contrast to TT, ADMA level is reported to be elevated among patients with cardiovascular problems and serves as the strongest risk predictor for coronary artery diseases based on in a review (Sibal, Agarwal, Home, & Boger, 2010). Very limited previous studies have investigated ADMA levels in ED patients with CAD, though a potential association has been demonstrated (Aktoz et al., 2010). A study conducted in Turkey participated by 92 men has shown a significantly higher ADMA level in patients with ED (0.75 ± 0.40 μmol/L) and CAD (0.50 ± 0.30 μmol/L) in comparison with control group without CAD or ED. This study also showed elevating ADMA levels as an independent risk factor for severity of ED, and ADMA may provide a possible link between CAD and ED (Aktoz et al., 2010). A similar result was obtained in a study of

132 elderly men performed by Maas (2005) which investigate the relationship between ADMA level, CAD and ED. The ADMA concentration level in the ED-CAD group (0.76 μmol/L, 95% CI: 0.65–0.91) are higher as compared to the ED-No-CAD group (0.49 μmol/L, 95% CI: 0.36–0.71) ($p < 0.001$) (Maas et al., 2005). Besides that, this study also reported ADMA as a strong independent predictor for presence of CAD in men with ED and the hazard ratio for getting CAD is 1.43 times higher with every 0.1 μmol/l increase in ADMA level (HR = 1.43, 95% CI: 1.2–1.7; $p < 0.001$) (Maas et al., 2005). These association studies between ADMA level, CAD and ED reflect a crucial relationship among them, suggesting ADMA levels could be a biomarker which is capable of integrating the presence of CAD in ED patients.

2.12.3 Hypertension

There are significant notable evidences that biomarkers especially TT and ADMA may play a part in deciding the incident of risk of hypertension especially in ED patients. A significant low TT level is recorded in hypertensive patients (230±77 ng/dL) as compared to control patients (343±92 ng/dL), $p<0.001$ in the study consisting of 49 men aged 40 years old and above (Ishikura, Asanuma, & Beppu, 2008). This result is in agreement with other studies which reported that hypertension patients have low levels of serum TT (Fogari et al., 2005; Fogari et al., 2002). Apart from these, a population-based study was conducted on 1484 Pomerania men aged 20 to 79 years old to investigate the association of risk of hypertension with TT levels. This study reported that men with low TT levels were 1.19 times more susceptible to have high risk of hypertension incident compared to men with higher TT concentrations (OR = 1.19, 95% CI: 1.10, 1.28) (Torkler et al., 2011). TT helps to increase the activity of eNOS which elevate the NO levels and lead to cGMP synthesis and induce more relaxed arteries which in turn lowered the blood pressure.

In contrast, ADMA is found to be high in hypertensive patients (Taner et al., 2013) and rats (Li, Guo, Chen, Hu, & Chen, 2011), resulting in increased mean blood pressure. A study conducted on association of ADMA level with hypertension showed that hypertensive patients had significantly higher levels of plasma ADMA (766 ± 217 nmol/l) than normal subjects without hypertension (393 ± 57 nmol/l) ($P < 0.001$). In this study the author reported that elevated ADMA levels are positively correlated with high mean blood pressure and may contribute to increased risk of hypertension (Wang, Strandgaard, Iversen, & Wilcox, 2009). This result was consistent with another study conducted in Gazi University Medical School on patients with Coronary Heart Diseases (CHD). Mean ADMA level is significantly higher in CHD patients with pulmonary arterial hypertension, (23.1 ± 9.2 μmol/L) compared to CHD patients without pulmonary arterial hypertension, (19.6 ± 7.4 μmol/L) and healthy control, n = 20, (17.1 ± 5.6 μmol/L) (Sanli et al., 2012). ADMA which is a NO synthase inhibitor is a significant predictor of morbidity. ADMA initiate inflammatory processes by interacting with inflammatory response proteins and affecting insulin sensitivity. Besides that, ADMA develop vascular damage which increase risk of high blood pressure in an individual (Perticone et al., 2010). Increasing serum ADMA and decreasing NO level was significantly associated with hypertension (Fan, Tsai, Hsu, Huang, & Tain, 2013).

2.12.4 Hyperlipidemia

Low serum TT is a risk factor for diabetes, CAD and hypertension including dyslipidemia or hyperlipidemia (Maggio & Basaria, 2009). A study conducted by Alqubaty on 35 men aged between 21 to 46 with normal to high lipid profile reported a significant reduction in serum TT level in hyperlipidemic patients (5.47 ± 1.85 nmol/L) compared to control group who had normal lipid profile (7.04 ± 1.83 nmol/L) ($p=0.026$). Apart from this, a negative correlation was observed between triglyceride

and serum TT level (R = -0.445, p = 0.007) (Alqubaty, 2014). Besides that, a population-based study on 1468 Pomeranian men aged 20 to 79 years old investigated the association of TT levels with lipid profile. This study found that low TT levels are significantly associated with higher total cholesterol (0.23 mmol/l, (95% CI, 0.02–0.42); and triglyceride concentrations (0.73 mmol/l, (95% CI, 0.53–0.94) (Haring et al., 2011). The decrease in TT level among obese and hypelipidemic patients might be because of degradation of testosterone in peripheral tissue into estrogens by the estrogen receptors in the male hypothalamus through a negative feedback mechanism (Alqubaty, 2014). However the exact effect of adverse lipid profile on lowering TT level was questionable and further studies should be conducted.

Scarce studies have been conducted on association of ADMA level with hyperlipidemia. The plasma concentration of ADMA is found to be significantly higher in hyperlipidemia patients. In a study conducted among dyslipidemia patients with mild CAD (n = 83, mean age 55.2 years), it is reported that a significant positive correlation was found between ADMA levels and total cholesterol ($r = 0.453$, $p < 0.0001$) and low-density lipoprotein cholesterol ($r = 0.482$, $p < 0.0001$). ADMA levels increased with increment in total cholesterol and low-density lipoprotein cholesterol levels. NO is an endothelium-derived vasoactive mediators which helps in regulating and maintaining the physiological vascular structure (Böger, 2006). However, ADMA is an endogenous inhibitor of NO-synthase and subsequently damage vascular tone (Siasos, Tousoulis, Antoniades, Stefanadi, & Stefanadis, 2007). The molecular mechanism explaining the increase of ADMA levels in hyperlipidemia patients is not completely understood. However, based on few pathophysiological evidences, it has proposed that oxidative stress could lead to elevation of ADMA levels (Ito et al., 1999). ADMA plays a crucial role in human endothelial function (Siasos et al., 2007). Studies demonstrate that gene expression and enzyme activity of protein arginine N-methyltransferase is increased in

the presence of LDL. Arginine N-methyltransferase is an enzyme which is responsible for ADMA synthase (Böger et al., 2000). Furthermore, high cholesterol level may degrade dimethylarginine dimethylaminohydrolase activity which eventually increases the bioavailability of ADMA. A low level of HDL was also positively associated with increased ADMA level due to decrease in NO synthesis (Lorin et al., 2013). Moreover, the presence of both high ADMA level and hyperlipidemia can result in a severe ED condition (Böger et al., 2009; Kurtoglu et al., 2014).

2.12.5 Lower Urinary Tract Symptoms/ Benign Prostatic Hyperplasia

Few studies have reported an increased risk of BPH or LUTS with low level of serum TT (Parsons, 2010). This observation infer that high TT level do not promote BPH associated with LUTS. Using the data from Prostate Cancer Prevention Trial (1993–2003), a case-control study was conducted on association of TT level with risk of BPH (n = 708). This study reported that men with high TT levels were 0.64 times more likely to have high risk of BPH incident (OR = 0.64, 95% CI: 0.43, 0.95, p = 0.04) (Kristal et al., 2008). In contrast, no studies to date have been reported on significant association of increased risk of BPH or LUTS with serum ADMA level.

2.12.6 Phosphodiesterase Type 5 Inhibitors (PDE5 Inhibitors)

Administration of sildenafil by ED patients with low TT (< 11.4 nmol/L) is significantly associated with increasing mean of TT up to 3.6 nmol/L ($p < 0.001$). This study by Spitzer proved that sildenafil could increase serum TT level in ED patients (Spitzer et al., 2013). In contrast, no changes are reported in ADMA level after treating ED patients with sildenafil (Sirmagul, Ilgin, Atli, Usanmaz, & Demirel-Yilmaz, 2013; Wierzbicki et al., 2006).

A strong positive correlation is found between vardenafil and ADMA ratio from baseline ($r = 0.80$; $P = 0.01$) at 540 minutes after vardenafil administration. In addition,

a significant reduction of -11.1% and -12.5 % in ADMA level are reported at 30 minutes ($p = 0.021$) and 45 minutes ($p = 0.002$) respectively after administration of vardenafil (Henrohn et al., 2015). However, there are no strong literature evidences to support the association of vardenafil with TT, although a few studies have been conducted to study the improvement of sexual function upon TT with vardenafil treatment (Van Der Made et al., 2009; Yassin, & Hammerer, 2014).

In the study conducted by Hellstrom, the mean of serum TT level increased after daily dose intake of 20 mg tadalafil for 9 months. The mean TT was significantly higher in the treatment group as compared to the placebo group ($p = 0.03$) (Hellstrom et al., 2008). No recent study is made on the association of ADMA with tadalafil.

2.13 Association of Lifestyle Factors with Biomarkers (TT & ADMA)

2.13.1 Physical Activities

A study on the effect of physical exercise on serum TT showed significant increase in TT level in 30 young men (aged 18 to 27 years old) who are actively involved in physical activities (pre exercise = 5.49±1.31 ng/dl vs after 12 weeks = 6.41±2.28 ng/dl $P<0.05$). This study also concluded that exercise or physical activities could increase serum TT level in men (Devi, Saxena, Rastogi, Goel, & Saha, 2014). This result is consistent with some other studies which have reported that exercise could increase testosterone level in physically active men (Dandona & Rosenberg, 2010; Vingren et al., 2010). A number of studies have been discussed on the mechanism of exercise in elevating the TT levels. Exercise reduces hepatic clearance of androgen by limiting hepatic blood flow and increasing TT levels in the body. Other than that, exercise regulates androgen receptors which are presents in skeletal and cardiovascular muscle and enhance these muscles reacting positively to androgenic hormones (Devi et al., 2014)

In contrary, synthesis of ADMA is found to be lowered with aggressive physical activity. Study conducted on Italian patients (n = 30) with mean age 52 ± 4.5 years reported ADMA level is significantly reduced after regular physical exercise (0.68 ± 0.06 µmol/L vs 0.48 ± 0.05 µmol/L; $p < 0.05$) (Riccioni et al., 2014). Another prospective cohort study found that plasma ADMA concentration is significantly decreased in 238 renal transplant patients after six months of exercise. ADMA concentration in the "exercising group" significantly decreased (3.5 ± 0.45 vs 2.11 ± 0.35 µmol/L, $P < 0.01$) and is also lower as compared to "non-exercising group" (2.11 ± 0.23 vs 3.25 ± 0.34 µmol/L, $P < 0.01$) (Teplan et al., 2014). The mechanisms of expressing the reduction of ADMA level caused by physical activities are not well explained. Synthesis of ADMA is heavily influenced by oxidative stress (Sydow & Münzel, 2003). Exercise was reported to reduce oxidative stress and eventually decrease plasma ADMA level (Choi et al., 2010). Furthermore, a recent study demonstrated that exercise could elevate mRNA gene expression of dimethylarginine dimethylaminohydrolase (DDAH), an enzyme which degrades ADMA synthase (Hanssen et al., 2011). Hence, increase in DDAH by physical exercise trigger reduction of ADMA level (Tanahashi et al., 2014).

2.13.2 Smoking

Many studies have found that smoking is one of the factors associated with severity of ED. But not many studies examined the role of smoking and biomarkers in ED patients. A few studies documented higher TT levels in smokers. Corona (2005) analysed 1,150 men attending outpatient clinic for sexual dysfunction and discovered higher TT levels in current smokers (17.8 ± 6.2 nmol/L) compared with non-smokers (16.3 ± 5.4nmol/L) after adjustment of confounding factors age and/or BMI (Corona et al., 2005). Besides, a cross sectional study carried out on 255 Brazilian men aged 30 to 70 years old also showed higher TT level in smokers (448.7 ± 153.4 ng/dL) compared

to non-smokers (438.7 ± 181.2 ng/dL) (Halmenschlager, Rossetto, Lara, & Rhoden, 2009). Even though there are numerous theories reporting the positive impact of cigarette smoking on production of sex hormone, few studies have demonstrated that smoking could help to decrease TT levels. A contrary result was reported on the effect of smoking on testosterone levels which showed that the plasma TT level of rats after being exposed to cigarette smoke for 60 min was lower than that of the control group (11.9 ± 4.1 nmol/L vs 13.7 ± 4.3 nmol/L, $p < 0.096$) (Park et al., 2012). The mechanism of cigarette smoking in influencing the male sex hormone levels is debatable. Nevertheless, several theories were put forth on how cigarette smoking may influence TT levels by various mechanisms. Beamud (2014) suggested that TT synthesis in smokers is interrupted by nicotine which inhibits luteinizing hormone (LH) secretion and cause DNA fragmentation and sequentially inhibits gonadotropin-releasing hormone (GnRH) (Beamud & Vidal, 2014). Inhibition of LH secretion prevents Leydig cells from secreting testosterone (Dong & Hardy, 2004) and subsequently lowers the TT level in smokers.

To our knowledge, the present study is unique which investigates the association between cigarette smoking and ADMA levels in ED patients since most of the previous studies are analysing non-ED patients. Schnabel analysed 1908 patients with risk of cardiovascular incident and found smoking status as the strongest predictor of increased ADMA level. This study reported that serum concentration of ADMA is significantly higher in current and ex-smokers (0.64 μmol/L) when compared to non-smokers (0.61 μmol/L, $p=0.002$) (Schnabel et al., 2005). Apart from this, a study conducted by Zhang *et al*. (2006) on 22 men reported a significant increase in serum ADMA level in smokers (1.10±0.54 μmol/L), who smoked at least 20 cigarettes per day compared to non-smokers (0.61±0.13 μmol/L) ($p=0.01$) (Zhang, Venardos, Chin-Dusting, & Kaye, 2006). Other than that, a cross sectional study that analysed 231 men aged 20 to 60

years reported that ADMA level in smokers were 6.4 times higher compared to non-smokers (6.4%, 95% CI: 0.4-11.3, p = 0.036). Smoking increases plasma ADMA concentration, with smoking status being reported as a significant predictor influencing ADMA levels (Meinitzer et al., 2007). Furthermore, significant reduction was reported in ADMA level after cessation of smoking (Baseline: 0.64 μmol/L, 5 weeks after cessation: 0.62 μmol/L, p = 0.003) (Puls et al., 2011). All of the above studies showed that ADMA levels were found to be higher in smokers than those in non-smokers. This could be because of the effects of cigarette smoke content on DDAH activity. Cigarette smoke down-regulated DDAH activity and might be primarily responsible for increase of ADMA level (Imamura et al., 2007; Sibal et al., 2010).

2.13.3 Alcohol Consumption

Apart from smoking, chronic alcohol intake can cause drastic changes in serum concentrations of certain biomarkers, which would result in ED. Maneesh and colleagues have observed a significant association between alcohol abuse and low plasma testosterone production in 46 male alcohol abusers aged 20 to 40 years old ($p < 0.001$). Serum testosterone level is negatively correlated with duration of alcohol abuse ($R^2 = 0.9518$, $P < 0.0001$) and was reported to be low in alcohol abusers (4.96±0.16 ng/ml) compared to non-alcoholics (7.56±0.13 ng/ml) (Maneesh, Dutta, Chakrabarti, & Vasudevan, 2006). Besides that, a 6.8% decrease in plasma concentration of TT is recorded in moderate alcoholics (95% CI, -1.0 – -12.5) (Sierksma et al., 2004) but significantly increases after reducing the dose of alcohol ($p < 0.05$) (Sarkola & Eriksson, 2003). In addition, an animal study also reported similar results where a high alcohol dosage induces a significant reduction of TT concentration in rats that prefer alcohol ($p = 0.001$) (Apter & Eriksson, 2003). These studies hypothesized that alcohol plays a significant role in modulating the TT level. Alcohol may alter the endocrine system and has an inhibitory effect on hypothalamic pituitary testicular axis which has

direct effect on testis. Alteration in gonadotropin releasing hormone receptor by alcohol may result in decrease of LH synthesis. Moreover, increased β-endorphin levels in heavy alcoholics may lead to testicular damage and diminish testosterone production (La Vignera et al., 2013).

In contrast, ADMA is found to be elevated in advanced alcoholic cirrhosis (1.12 ± 0.08 µmol/l) as compared to control group (0.58±0.07 µmol/l, $p<0.05$) (Lluch et al., 2004). Patients who participated in this study had a history of alcohol consumption >80 g/day for at least 5 years and reported having liver cirrhosis. Liver plays an important role in the metabolism of ADMA. ADMA is degraded by DDAH activity which mainly occurs in the liver. The degradation of enzyme DDAH in the liver due to hepatocellular damage would increase the ADMA levels (Lluch et al., 2004). These elevated ADMA levels diminish NO production which eventually leads to erectile problem among men. However, the exact effect of alcohol on metabolism of ADMA has not been well documented.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study Design and Setting

A cross-sectional prospective study was conducted in patients who attended ED, surgery, eye or medical clinics in a university hospital, University Malaya Medical Centre (UMMC) at Kuala Lumpur, Malaysia from September 2013 until April 2015. The study was conducted in accordance with the Declaration of Helsinki and was approved by the medical ethics committee of the UMMC (MEC reference number 1010.18). Sample size was calculated from the published guidelines for testing individual predictors (VanVoorhis & Morgan, 2007). Based on the study, regression sample size for testing individual predictors is determined using formula, $N > 104 + m$, (where m is the number of predictors or independent variables). Convenience sampling was applied to select the subjects for this study (Huri, Sanusi, Razack, & Mark, 2016). A random selection of patients from the non-ED pool was included in the study (controls). Patients aged 40 years old and above (Pastuszak, 2014) who met the inclusion criteria were approached and briefed about the purpose of this study. Those who fulfilled the inclusion-exclusion criteria were selected and patient information sheet (PIS) was given to them. Patients who voluntarily agreed to participate in this study were given a consent form to acknowledge the agreement to participate in this study. A face-to-face interview was conducted to complete a study questionnaire. An overview of the study methodology is illustrated in Figure 3.1.

3.2 Inclusion and Exclusion Criteria

(a) ***Inclusion Criteria (case)***

- Adult male patients ≥ 40 years old (Pastuszak, 2014)
- Patients who have IIEF-5 score ≤ 21 (Pastuszak, 2014)

(b) ***Inclusion Criteria (control)***

- Adult male patients ≥ 40 years old
- Patients who have IIEF-5 score > 21

(c) ***Exclusion Criteria (case)***

- Patients that are critically ill who required medical treatment
- Patients who are unwilling to participate in the survey
- Patients who are diagnosed with psychiatry illness or mentally challenged
- Patients who underwent surgical treatment for ED
- Patients who are admitted and required intervention in hospital
- Patients who received non-pharmacological local therapy (herbal medications)
- Patients who received hormone therapy for ED (hypogonadism)
- Immunocompromised patients (HIV, AIDS and cancer patients)

(d) ***Exclusion Criteria (control)***

- Patients that are critically ill requiring medical treatment
- Patients who are unwilling to participate in the survey
- Patients who are diagnosed with psychiatry illness or mentally challenged
- Patients who are admitted and required intervention in hospital
- Immunocompromised patients (HIV, AIDS and cancer patients)

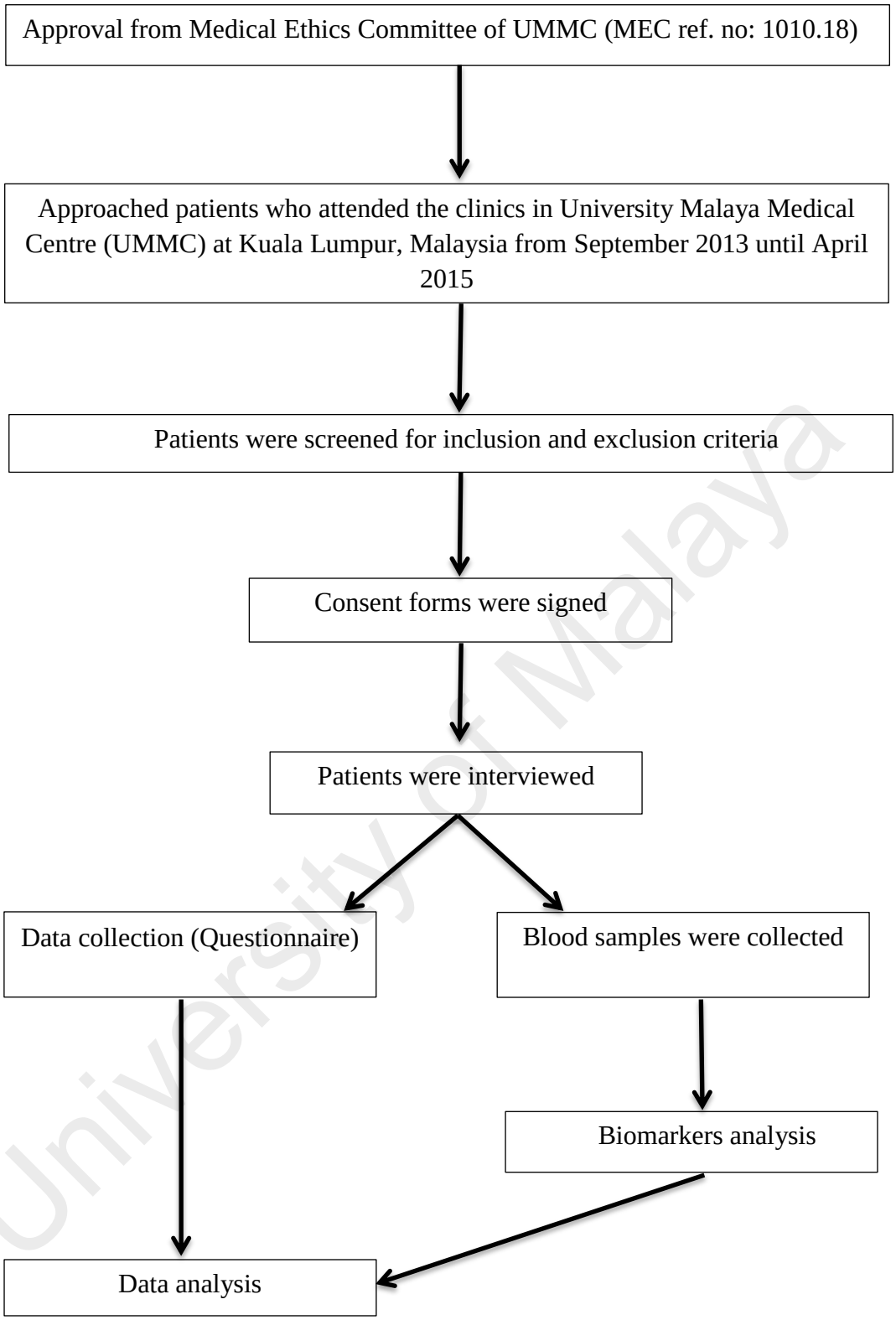

Figure 3.1: Overview of Study Methodology.

3.3 Sample Size

In this study, the minimum sample size was calculated from the published guidelines to test individual predictors (Green, 1991; VanVoorhis & Morgan, 2007). According to the study, regression sample size for testing individual predictors is determined using a formula, N > 104 + m, (where m is the number of predictors or independent variables). There were 22 variables involved in each binary logistic regression analysis. Therefore, based on the above references; the minimum sample size calculated was 126 per arm. In this study, the number of samples recruited (n = 138) was more than the minimum number of sample required. The formula of sample size calculation is as shown below:

N > 104 + m, (m = number of predictor variables)

104 + (22) = 126 (the minimum number of samples required)

3.4 Questionnaire

Patients who gave their consent were provided with a study questionnaire which was adapted from a few previous studies (Ab Rahman et al., 2011; Khoo et al., 2008; Tan et al., 2007). The questionnaire was divided into three parts. The first part (Part A) of the questionnaire assessed the socio-demographic characteristics such as age, ethnicity, marital status, occupation, academic qualification and monthly income. The second part of the questionnaire measured life style factors of patients which comprised of factors such as physical activities (intensity and duration of physical activities), smoking history (intensity and duration of smoking) and alcohol consumption. Part C analysed detailed medical history (history of comorbidities), sexual health (IIEF-5) and list of PDE-5 inhibitors consumed by ED patients. The outline of the study questionnaire is as follow.

3.4.1 Assessment of Demographic Factors (Part A)

Socio-demographic characteristics were assessed in this first part of the questionnaire. Patient's age was calculated using year of birth as stated in hospital information system. Ethnic is classified into three major populations of Malaysia; Malays, Chinese and Indians. Marital status is categorized into married and others (never married or single, divorced, and widower). Occupation is classified into 5 categories; government employee, non-government employee, self-employed, pensioner and unemployed. Respondents' academic qualification is measured based on Malaysian education system which includes no formal schooling, primary education, secondary educations (PMR, SPM, STPM, A-Level, certificate) and tertiary/higher educations (Diploma, Bachelor degree and Postgraduate degree) (Ab Rahman et al., 2011; Momtaz et al., 2014). Meanwhile monthly income is defined as low (< RM1000), medium (RM 1000 – RM 2999) and high (RM ≥ 3000) based on patients' salary in Malaysian Ringgit (MYR) (Ab Rahman et al., 2011; Momtaz et al., 2014).

3.4.2 Assessment of Lifestyle Factors (Part B)

This part of questionnaire evaluates lifestyle factors of patients including items on physical activity, cigarette smoking history, and alcohol consumption. Physical activities was assessed on how often patients involved in activities which took hard physical efforts that made them breather harder than normal in the past 3 years (Ab Rahman et al., 2011). Among the other questions asked to assess physical activities are:

- Over the last 3 years, how often did you do physical activities? For example, heavy lifting, aerobic, carrying light loads, bicycling at a regular pace, or playing badminton.
- How many days per week were usually spent on doing these physical activities?
- How much total time did you usually spend on doing physical activities on one of those days?

The smoking history of patients was assessed. A patient was considered a smoker if he had smoked more than one cigarette per day for at least one year; an ex-smoker if he had stopped smoking more than one year from the day of interview and a non-smoker if he had never smoked cigarette in his whole life (Chrysohoou et al., 2013). Smokers were asked additional questions on how many cigarettes they smoked per day and the number of years they have been smoking. Based on the questionnaire responses, the respondents' status is classified as current smoker, non-smoker, and ex-smoker with varying intensity of smoking (number of cigarettes per day) and duration of smoking (years of smoking) (Gades et al., 2005). Patients were evaluated based on consumption of drinks such as beer, wine, spirit, samsu, tuak, sake or others which contain alcohol (Ab Rahman et al., 2011). Response categories ranged from no (never drank alcohol in his life) to alcoholic (with an average of daily or weekly alcohol consumption) and ex-alcohol consumer (had stopped drinking alcohol more than one year from the day of interview) (Ab Rahman et al., 2011; Bacon et al., 2006).

3.4.3 Assessment of Clinical Factors and Sexual Health (Part C)

Self-reported health conditions of patients with history of comorbidities were questioned in this questionnaire. The information on comorbidities was obtained from patients' medical records. Meanwhile, the list of PDE-5 inhibitors prescribed for ED patients was obtained from Pharmacy Information System, UMMC using patients' RN number. The severity of ED was measured using the International Index of Erectile Function-5 (IIEF-5) which is a self-evaluation questionnaire used for the assessment of male sexual function including screening, diagnosis of severity of ED (Pastuszak, 2014). Participants were asked to rate their ability to achieve and maintain an erection that is good enough for a satisfactory sexual intercourse without any treatment for the past 6 months. Response options ranged from very low, low, moderate, high and very high. Among the questions asked were as follow:

- How do you rate your confidence that you could get and keep an erection?
- When you had erections with sexual stimulation, how often were your erections hard enough for penetration?
- During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?
- During sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?
- When you attempted sexual intercourse, how often was it satisfactory for you?

Presence of ED is denoted by IIEF score of equal or less than 21. Severity of ED is further categorised based on the IIEF scores: severe (5-7), moderate (8-11), mild to moderate (12-16), mild (17-21), and no ED (22-25). For analysis purposes, a binary variable was created based on IIEF-5 where scores from 12-21 is considered "less severe (Mild to Mild/Moderate)" and 5–11 is considered as "moderate to severe" ED. The IIEF-5 assessments were completed by consultant urologists in UMMC with a specialisation in ED.

3.5 Blood Sampling and Biomarkers Analysis

Ten (10.0) ml of blood samples were collected in two SST tubes (BD vacutainer) and were immediately centrifuged at 4000 rpm for 15 min (Önder et al., 2014). The supernatant (serum) was decanted into clean eppendorf centrifuge tubes and kept in the -20°C freezer (Remi scientific, Malaysia, model: DW-YL270) until analysis. ADMA and TT levels were determined by using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to manufacturers' instructions (Uscn Life Science Inc, Wuhan, China) (Ali et al., 2012; Griffeth, Carretero, & Burks, 2013).

3.6 Laboratory Analysis for Biomarkers

ELISA is a quantitative gold standard immunoassay used for the selective detection of targeted antigens with a measurable substrate.

Briefly, micro plates pre-coated with monoclonal antibody specific to the respective biomarkers were used for this research. Respective antigen biomarkers present in standards and samples were bound to immobilize antibodies in the micro plate. The remnants of unbound antibodies in the micro plate were washed away. The detection reagent (A) was added to the complete set of antigen-antibody in the micro plate. The remnants of unbounded detection reagent (A) in the micro plate were washed away. Addition of 3,3', 5,5''-tetramethylbenzidine (TMB) peroxidase substrate was used for the detection of reagent (A) in the micro plate. Development of blue colour occurred after incubation period which was proportional with the amount of antigen presence in micro plate wells. Addition of stop solution changed the blue solution to yellow and the intensity of the colour was measured at 450nm using a Tecan infinite micro plate reader (Mnnedorf, Switzerland) (Aydin, 2015; Hnasko, Lin, McGarvey, & Stanker, 2011).

3.6.1 Reagents and Materials Used for ELISA

Commercially available ELISA kits were purchased from Uscn Life Science Inc, Wuhan, China (Ali et al., 2012; Griffeth et al., 2013), which contain reagents and materials as below:

- Standard: A calibration substance with preservatives.
- Standard Diluent: 2% Bovine Serum Albumin (BSA), 0.02% sodium azide
- Detection Reagent A: biotin-conjugated analyte
- Assay Diluent A: TBS with 1% BSA and 0.01% sodium azide
- Detection Reagent B: Horseradish Peroxidase (HRP)-linked Avidin, 50% glycerol
- Assay Diluent B: TBS with 1% BSA and 0.01% sodium azide
- Stop Solution: 1mol/L H_2SO_4
- TMB Substrate: 0.05% 3,3’,5,5’ tetramethylbenzidine

- 30X Wash Buffer: 30X Tris Buffered Saline (TBS)
- Antibody coated microwells (96-well strip plate)
- Plate sealer for 96 wells

3.6.2 Equipment and Materials Used for ELISA

- Hettich Zentrifugen centrifuge (Tuttlingen, Germany)
- Tecan infinite micro plate reader with 450 ± 10nm filter (Männedorf, Switzerland)
- Micro plate shaker
- Binder forced convection incubator (37 °C) (Tuttlingen, Germany).
- Thermoscientific single and multi-channel pipettes (Finland)
- Disposable pipettes tips, eppendorf tube (autoclaved), racks, distilled & deionised water, absorbent & tissue paper, empty container for wash solution, beaker (100ml), biohazard bags to dispose wastes, gloves, reservoir (for multichannel pipette), table cover, and alcohol (to disinfect the table)

3.6.3 Reagent Preparation

All ELISA kits' components and samples were brought to room temperature before use. One (1.0) mL of standard diluent was reconstituted with standard to prepare 1,000 ng/mL of stock solution. Five eppendorf tubes were prepared with 0.6 mL of standard diluent. A triple dilution series was conducted to produce 5 points of diluted standard; 1000 ng/mL, 333.33 ng/mL, 111.11 ng/mL, 37.04 ng/mL, 12.35 ng/mL, and the last tube with concentration 0 ng/mL as the blank. Detection reagent A (120 µL) was reconstituted with 12mL of assay diluent A in the ratio of 1:100 and kept for 10 minutes at room temperature. The same procedure was applied for detection reagent B. A wash solution of 600mL was prepared by diluting 20mL of wash buffer concentrate (30×) with 580mL of deionized and no dilution was applied for TMB substrate.

3.6.4 Sample Preparation

The frozen serum samples were thawed to room temperature before use. Repeatedly freezing and thawing were avoided to prevent protein degradation and denaturalization.

3.6.5 Assay Procedure

Firstly, the wells for diluted standards, blank and samples were determined. Five wells for standard points and one well for blank were prepared. Fifty (50) µL of each dilution of standard, blank and samples were placed into the appropriate wells respectively. Then, 50µL of detection reagent A were added into each well immediately using multi-channel pipettes and then covered with a plate sealer. The plate was shaken gently using a micro plate shaker for few min. The plate was incubated for 1 hour at 37°C (body temperature). After incubation, the solution inside the plate was aspirated and washed with 350µL of wash solution. The remaining liquid from all wells was removed completely by snapping the plate onto absorbent paper. This procedure was repeated 3 times. Next, 100µL of detection reagent B were added into each well. The plate was covered with plate sealer and incubated for 30 minutes at 37°C. Then, the aspiration and wash process were repeated 5 times. After the washing process, 90µL of TMB substrate were added into each well and covered with a new plate sealer. This procedure was protected from light. The liquid turned into blue colour by the addition of TMB substrate solution. The plate was incubated for 15 - 25 minutes at 37°C. Finally, 50µL of stop solution was added into each well. The liquid turned yellow upon the addition of stop solution. Water droplets and fingerprints on the bottom of the plate were removed using clean tissue papers. Absorbance level was measured using a microplate reader (Tecan infinite M200) at 450 nm. All samples, controls and standards were analysed in duplicate.

3.7 Statistical Analysis

The data was analysed using Statistical Package for Social Sciences (SPSS) version 19.0 (IBM Corp. Released 2010, IBM SPSS Statistics for Windows, version 19.0. Armonk, NY: IBM Corp, USA). Descriptive statistics were used to summarize patients' demographic, clinical and lifestyle characteristics. Discrete variables were expressed as frequencies and percentages (N, %). On the other hand, continuous data was expressed as mean ± standard deviation. Chi-square test was used to analyse discrete variables, meanwhile one-way ANOVA was used to compare the means of continuous variables. "Confounding variables" could influence both the dependent and independent variable. These confounding variables should be controlled to reduce the effect on an observational study. Adjustments of confounding variables can be made in the data analysis so that the effects of confounder would be removed from the final results. Fisher's exact test will be used when one or more of the cells have an expected frequency of five or less. Continuity correction chi-square will be applied for 2 x 2 tables meanwhile pearson chi square test will be used for variable consist of two or more categorical/independent groups. Correlations were analysed using the parametric Pearson's correlation coefficient (for continuous variables). All results with $p<0.05$ were considered as statistically significant. A correlation coefficient of zero indicates that there is no linear relationship prevailing between two continuous variables, and a correlation coefficient of ±1 indicates a strong linear relationship. The correlation is strong enough if the correlation coefficient is closer to ±1. The two continuous variables are directly related if the coefficient is a positive number, and inversely related if the coefficient is a negative number (Mukaka, 2012). To build the predictor model for severity of ED, all variables that showed significant association with both primary analysis (case & control group) and secondary analysis (severity of ED, TT and ADMA) were subjected to binary logistic regression test (enter method) to determine

their odds ratio (OR) and confidence intervals. Hosmer and Lemeshow test with $p > 0.05$ indicates that the entered data is suitable with the tested model. Nagelkerke R square (R^2) value shows the percentage of predictors in influencing dependent variable. The receiver-operating characteristic (ROC) curve was used to determine the cut-off point for biomarker levels (ADMA, TT). The ranking of predictors in the model were determined by the odds ratio (OR) values. Results with $p < 0.05$ were considered statistically significant.

3.7.1 Predictor Models

3.7.1.1 Predictor Models for Severity of Erectile Dysfunction

Binary logistic regression test (enter method) was used to build the predictor model for severity of ED. The dependent variable for this modelling is severity of ED with scale 1: participants with IIEF scores of 12-21 and 2: participants with IIEF scores of 5–11. Variables which gave significant association ($p<0.05$) with case & control group (primary analysis) and severity group (secondary analysis) were subjected to this binary logistic regression test.

3.7.1.2 Predictor Models for Total Testosterone (TT)

Binary logistic regression test (enter method) was used to build the predictor model for TT. The ROC analysis indicated TT cut-off point of 9.55 nmol/L. The dependent variable for this modelling is TT with scale 1: high serum concentration level of TT, ≥ 9.55 nmol/L and 2: low serum concentration level of TT < 9.55 nmol/L. Variables which gave significant association ($p<0.05$) with both case and control group (primary analysis), and TT level (secondary) were subjected to this modelling.

3.7.1.3 Predictor Models for Asymmetric Dimethylarginine (ADMA)

Binary logistic regression test, enter method was used to build the predictor model for ADMA for Malaysian ED patients. An ADMA cut-off point of 0.665 µmol/L

yielded from ROC analysis. The dependent variable for this modelling is ADMA with scale 1: high concentration of serum ADMA, ≥ 0.665 µmol/L and 2: Low concentration of serum ADMA, < 0.665 µmol/L. Variables which gave significant association ($p<0.05$) at both primary and secondary analysis were subjected to this binary logistic regression test.

CHAPTER 4: RESULTS

4.1 Demographic, Clinical and Lifestyle Characteristics of Study Population

A total of 337 patients were approached. Out of these, 42 respondents were excluded as they do not fulfill the inclusion criteria while 19 respondents disagreed to participate in this study. As a result, 276 patients who met the inclusion-exclusion criteria were participated in this study whereas 138 patients were reported having ED (IIEF scores ≤ 21) and categorized as the case group. The balance 138 patients who have IIEF > 21 were categorized as the control group. The distribution of patients is summarized in Figure 4.1. The details of demographic, clinical and lifestyle characteristics of both case and control groups were shown in Tables 4.1, 4.2 and 4.3 respectively.

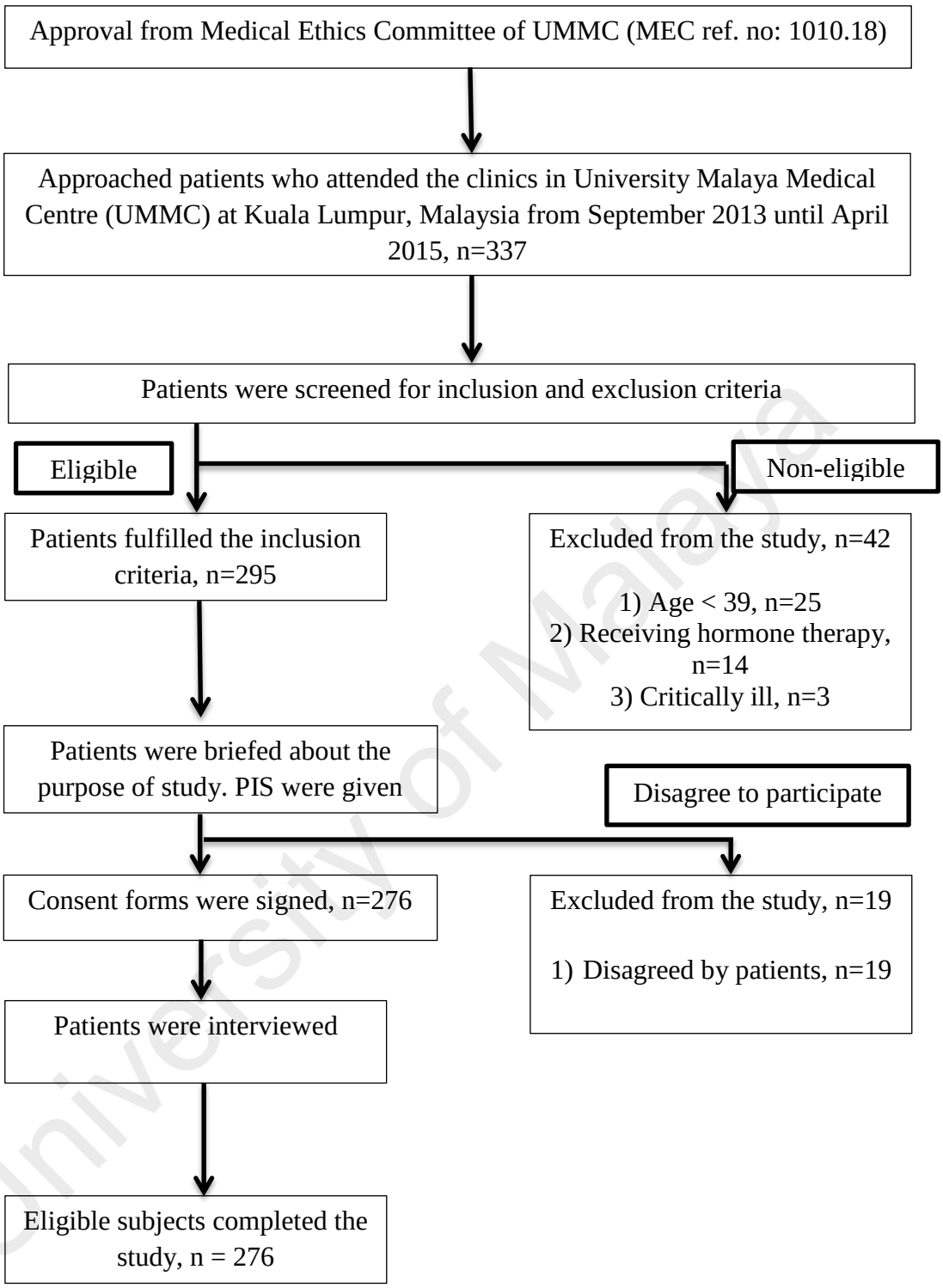

Figure 4.1: Flow Chart of Patient Disposition

Table 4.1: Demographic Characteristics of Subjects (n = 276)

Demographic factors	Case n=138 (%)	Control n=138 (%)	p Value
Ethnicity			
Malay	68 (49.3)	64 (46.4)	0.890[a]
Chinese	39 (28.3)	41 (29.7)	
Indian	31 (22.5)	33 (23.9)	
Marital Status			
Married	133 (96.4)	138 (100)	0.06 [b]
Others	5 (3.6)	0 (0.00)	
Occupation			
Government Employee	36 (26.1)	65 (47.1)	< 0.001[a] *
Non-government employee	29 (21.0)	11 (8.0)	
Self Employed	21 (15.2)	7 (5.1)	
Pensioner	30 (21.7)	34 (24.6)	
Unemployed	22 (15.9)	21 (15.2)	
Academic Qualification			
Primary	11 (8.0)	9 (6.5)	0.006 [a] *
Secondary	111 (80.4)	92 (66.7)	
Tertiary	16 (11.6)	37 (26.8)	
Monthly Household Income			
Low	30 (21.7)	30 (21.7)	< 0.001[a] *
Medium	65 (47.1)	24 (17.4)	
High	43 (31.2)	84 (60.9)	

[a] Computed using Pearson Chi Square; [b] Computed using Fisher's Exact Test;
* statistically significant ($p<0.05$).

Table 4.2: Clinical Characteristics of Subjects (n = 276)

Clinical factors	Case n=138 (%) / Mean ± SD	Control n=138 (%) / Mean ± SD	p Value
Type 2 Diabetes Mellitus			
Present	70 (50.7)	73 (52.9)	0.810[a]
Absent	68 (49.3)	65 (47.1)	
Hypertension			
Present	85 (61.6)	77 (55.8)	0.392[a]
Absent	53 (38.4)	61 (44.2)	
Hyperlipidemia			
Present	65 (47.1)	56 (40.6)	0.332[a]
Absent	73 (52.9)	82 (59.4)	
Coronary Artery Disease			
Present	80 (58.0)	72 (52.2)	0.397[a]
Absent	58 (42.0)	66 (47.8)	
Lower Urinary Tract Symptoms/ Benign prostatic hyperplasia			
Present	42 (30.4)	35 (25.4)	0.421[a]
Absent	96 (69.6)	103 (74.6)	
Total Testosterone (TT) (nmol/L)	11.58 ± 2.53	20.94 ± 2.89	< 0.001[b] *
Asymmetric Dimethylarginine (ADMA) (µmol/L)	0.65 ± 0.16	0.50 ± 0.08	< 0.001[b] *

[a] Computed using Continuity Correction; [b] Computed using one way anova test; *statistically significant ($p<0.05$).

Nominal data are reported as number (percentage), whereas interval data are expressed as mean±SD.

Table 4.3: Lifestyle Characteristics of Subjects (n = 276)

Lifestyle Factors	Case n=138 (%)	Control n=138 (%)	p value
Physical activities			
Yes	48 (34.8)	83 (60.1)	< 0.001[c] *
No	90 (65.2)	55 (39.9)	
Duration of physical activities			
No	90 (65.2)	55 (39.9)	< 0.001[a] *
≤ 2 days/week	31 (22.5)	25 (18.1)	
> 2 days/week	17 (12.3)	58 (42.0)	
Intensity of physical activities			
No	90 (65.2)	55 (39.9)	< 0.001[a] *
< 1 Hour/day	15 (10.9)	41 (29.7)	
≥ 1 Hour/day	33 (23.9)	42 (30.4)	
Smoking			
Yes	81 (58.7)	57 (41.3)	0.006 [c] *
No	57 (41.3)	81 (58.7)	
Duration of smoking			
No smoking	57 (41.3)	81 (58.7)	< 0.001[a] *
1 – 20 years	8 (5.8)	29 (21.0)	
21 – 40 years	42 (30.4)	18 (13.0)	
> 40 years	31 (22.5)	10 (7.2)	
Intensity of smoking			
No Smoking	57 (41.3)	81 (58.7)	< 0.001[b] *
1 – 2 Cigarette(s)/day	3 (2.2)	23 (16.7)	
3 – 5 Cigarettes/day	68 (49.3)	25 (18.1)	
≥ 6 Cigarettes/day	10 (7.2)	9 (6.5)	
Alcohol consumption			
Yes	41 (29.7)	29 (21.0)	0.128 [c]
No	97 (70.3)	109 (79.0)	

[a] Computed using Pearson Chi Square; [b] Computed using Fisher's Exact Test;
[c] Computed using Continuity Correction; *statistically significant (p<0.05).

4.2 Severity of Erectile Dysfunction (IIEF-5 scores)

Out of the 276 men who completed the study, 138 (50.0%) reportedly had IIEF-5 scores less than or equal to 21 (≤ 21). The mean IIEF score of ED patients is 13.80 ± 3.80. Approximately 26.1% of case group were having mild ED. Half of them were with mild to moderate ED. Around 15.2% had moderate ED whereas about 8.7% subjects with severe ED. Meanwhile, the mean IIEF score of control group (> 21) is 23.12 ± 1.39, n=138. The erectile function scores of the ED patients (case group) were shown in Figure 4.2.

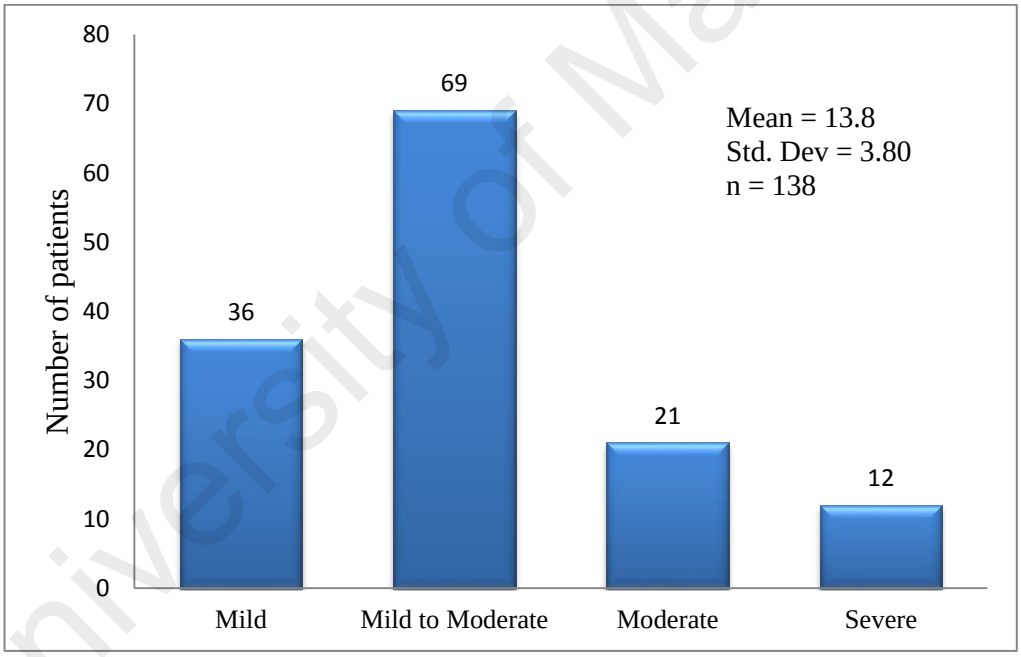

Figure 4.2: Severity of ED

4.3 Biomarkers

ADMA levels were significantly higher in ED patients (0.65 ± 0.16 vs 0.50 ± 0.08 µmol/L; P <0.01). On the contrary, TT levels were significantly greater in the control group compared to ED patients (20.94 ± 2.89 vs 11.58 ± 2.53 nmol/L; P <0.01). There was a significant negative correlation between serum TT and ADMA levels in the ED population (r = -0.680; P <0.01). Figures 4.3 and 4.4 show TT and ADMA level of control versus case group respectively.

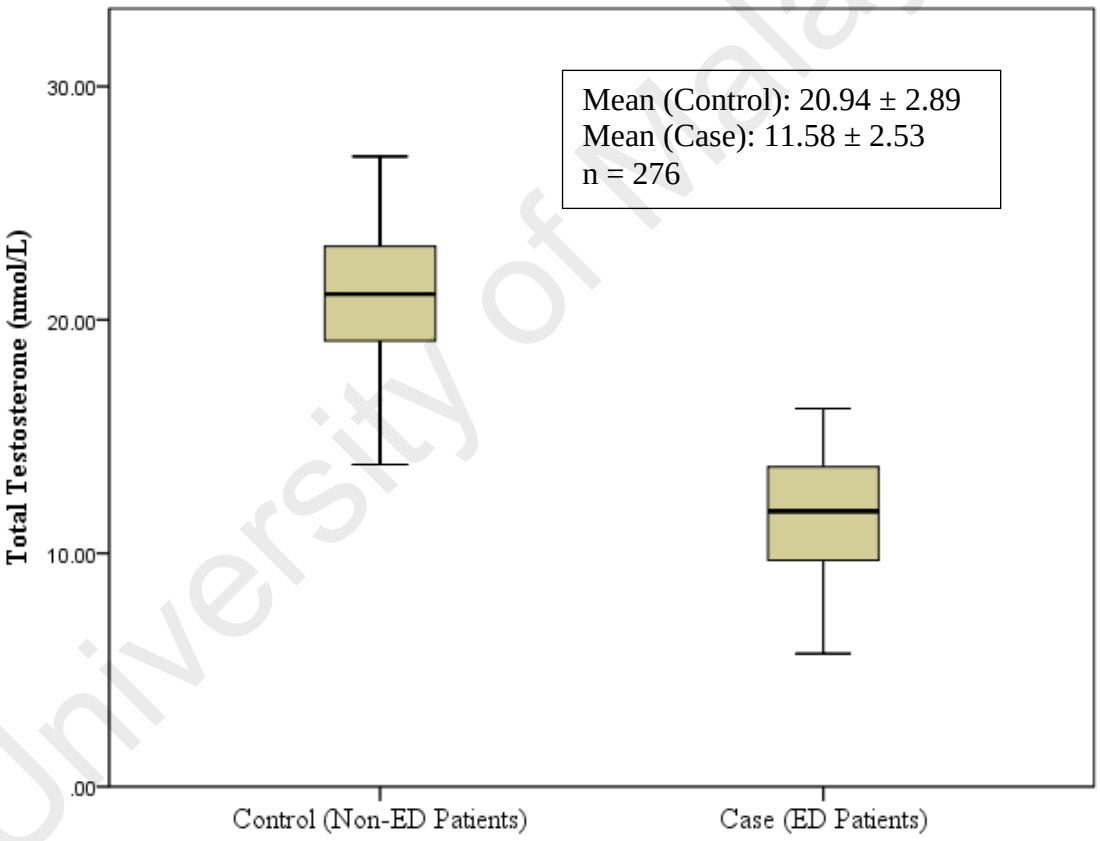

Figure 4.3: TT level (nmol/L) of control versus case group

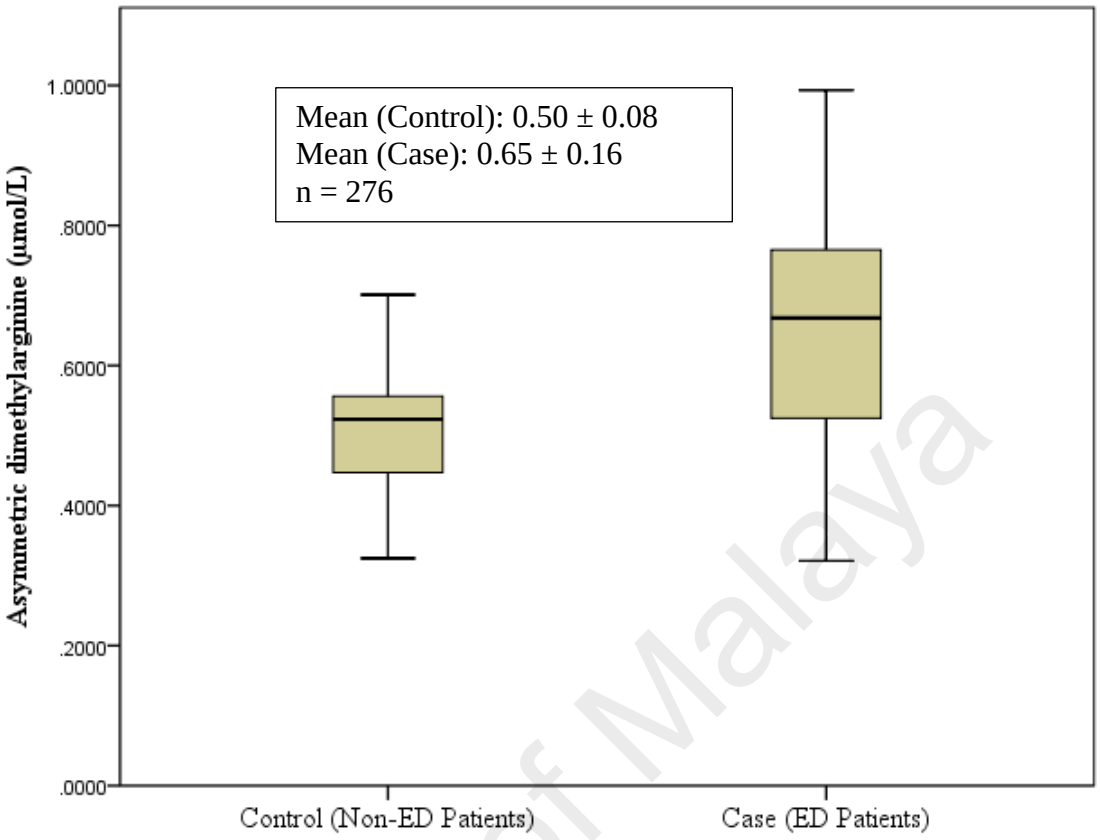

Figure 4.4: ADMA level (µmol/L) of control versus case group

4.4 Normality Tests for IIEF Scores and Biomarkers (TT and ADMA)

The data distributions for IIEF scores, TT and ADMA levels were tested using tests such as Shapiro-Wilks test and Kolmogorov-Smirnov test. The results showed that the IIEF scores, TT and ADMA levels were normally distributed. The values of skewness and kurtosis were within the range of ± 1.96. The results of all above mentioned tests concluded that the data of both IIEF scores and biomarkers were normally distributed. The normality tests results are presented in Table 4.4.

Table 4.4: Normality Test Result for IIEF Scores and Biomarkers (TT and ADMA)

	IIEF scores	TT	ADMA
Skewness	-0.384	-0.168	0.058
Standard error of skewness	0.206	0.206	0.206
Kurtosis	-0.482	-0.827	-0.525
Standard error of kurtosis	0.410	0.410	0.410
Shapiro-Wilks test	0.968	0.087	0.242
Kolmogorov-Smirnov test	0.092	0.177	0.200

4.5 IIEF Scores versus TT and ADMA

Pearson correlation coefficient test was computed to assess the correlation between the IIEF scores with TT and ADMA. The strength of association between IIEF scores and serum TT level was found to be positively high and significant ($r = 0.741$, $p = 0.001$), whereas a strong inverse correlation was recorded between IIEF scores and serum ADMA level, ($r = - 0.629$, $p = 0.001$). The correlation plots of IIEF scores versus TT and ADMA were presented in Figures 4.5 and 4.6 respectively.

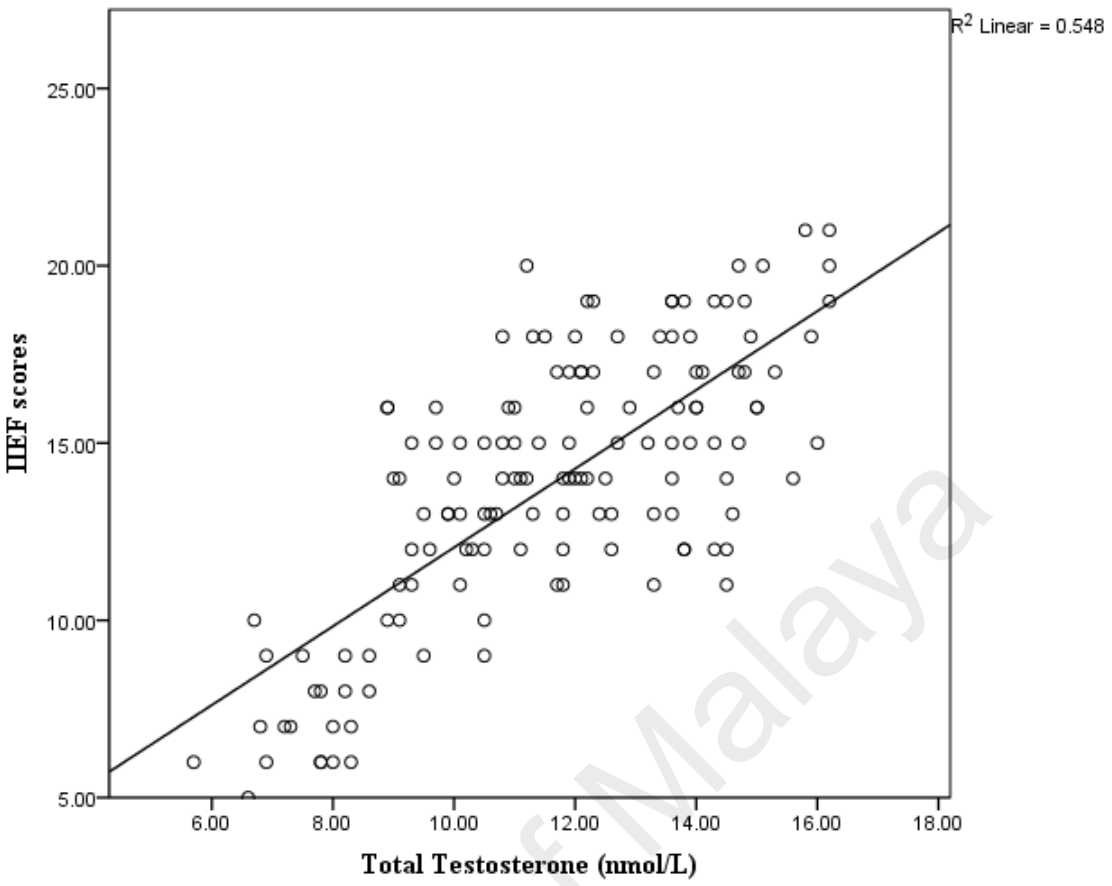

Figure 4.5: IIEF scores versus TT levels

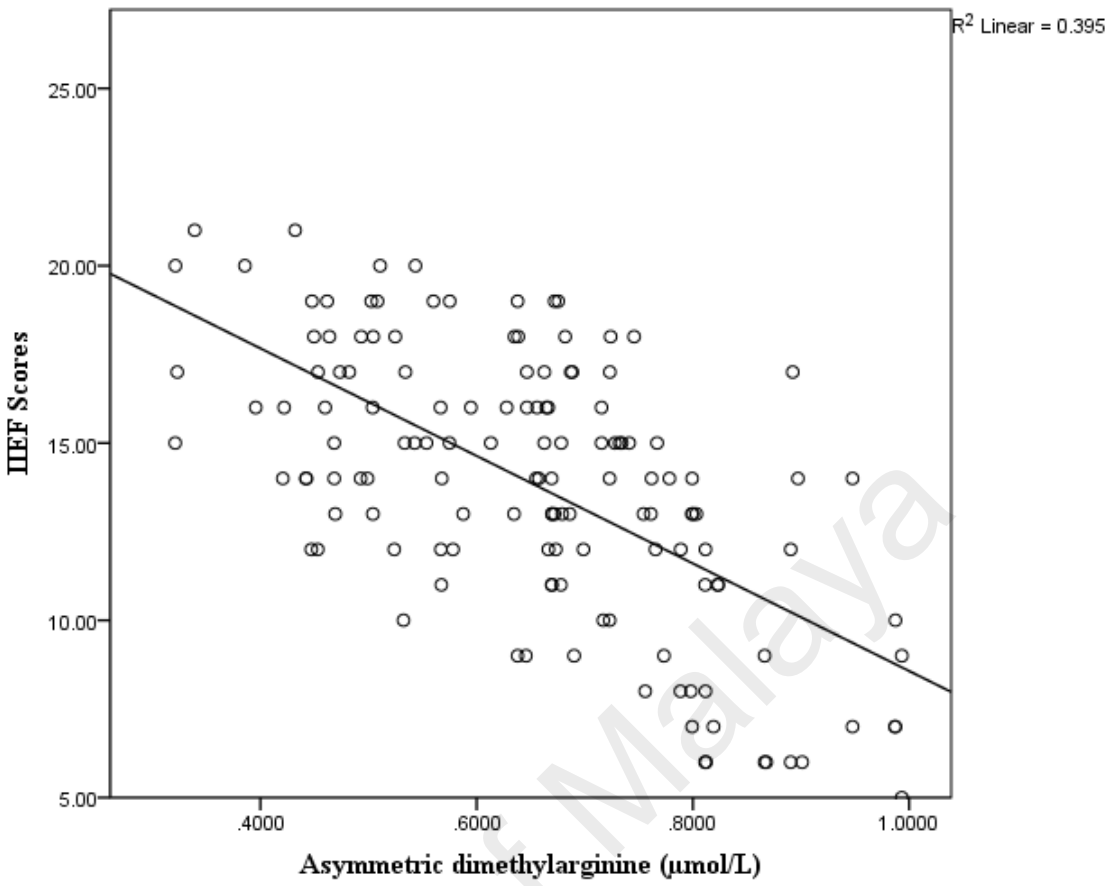

Figure 4.6: IIEF scores versus ADMA levels

4.6 Association of Demographic, Clinical and Lifestyle Factors with Severity of ED

A total of 138 patients who have IIEF scores ≤ 21 were subjected to this association study. The association of ED severity subgroup (mild to mild/moderate and moderate to severe) with demographic, clinical and lifestyle characteristics were presented in Tables 4.5, 4.6 and 4.7 respectively.

Table 4.5: Association of Demographic Factors with Severity of ED (n = 138)

Demographic factors	Mild to Mild/Moderate n=105 (%)	Moderate to Severe n=33 (%)	p Value
Ethnic			
Malay	52 (49.5)	16 (48.5)	0.951[a]
Chinese	29 (27.6)	10 (30.3)	
Indian	24 (22.9)	7 (21.2)	
Marital Status			
Married	105 (100.0)	28 (84.8)	0.001[b] *
Others	0 (0.0)	5 (15.2)	
Occupation			
Government Employee	33 (31.4)	3 (9.1)	< 0.001[b] *
Non-government employee	26 (24.8)	3 (9.1)	
Self Employed	17 (16.2)	4 (12.1)	
Pensioner	16 (15.2)	14 (42.4)	
Unemployed	13 (12.4)	9 (27.3)	
Academic Qualification			
Primary	4 (3.8)	7 (21.2)	0.006 [b] *
Secondary	88 (83.8)	23 (69.7)	
Tertiary	13 (12.4)	3 (9.1)	
Monthly Household Income			
Low	13 (12.4)	17 (51.5)	< 0.001[b] *
Medium	51 (48.6)	14 (42.4)	
High	41 (39.0)	2 (6.1)	

[a] Computed using Pearson Chi Square; [b] Computed using Fisher's Exact Test; * Statistically significant ($p<0.05$).

Table 4.6: Association of Clinical Factors with Severity of ED (n = 138)

Clinical factors	Mild to Mild/Moderate n=105 (%)	Moderate to Severe n=33 (%)	p Value
Type 2 Diabetes Mellitus			
Present	51 (48.6)	19 (57.6)	0.482[a]
Absent	54 (51.4)	14 (42.4)	
Hypertension			
Present	66 (62.9)	19 (57.6)	0.735[a]
Absent	39 (37.1)	14 (42.4)	
Hyperlipidemia			
Present	46 (43.8)	19 (57.6)	0.237[a]
Absent	59 (56.2)	14 (42.4)	
Coronary Artery Disease			
Present	58 (55.2)	22 (66.7)	0.338[a]
Absent	47 (44.8)	11 (33.3)	
Lower Urinary Tract Symptoms/ Benign Prostatic Hyperplasia			
Present	26 (24.8)	16 (48.5)	0.076[a]
Absent	79 (75.2)	17 (51.5)	
Total Testosterone (TT) (nmol/L)	12.49 ± 1.96	8.70 ± 1.95	< 0.001[b] *
Asymmetric Dimethylarginine (ADMA) (µmol/L)	0.61 ± 0.14	0.80 ± 0.12	< 0.001[b] *

[a] Computed using Continuity Correction; [b] Computed using one way anova test; * Statistically significant ($p<0.05$).

Nominal data were reported as number (percentage), meanwhile interval data are expressed as mean±SD.

Table 4.7: Association of Lifestyle Factors with Severity of ED (n = 138)

Lifestyle Factors	Mild to Mild/Moderate n=105 (%)	Moderate to Severe n=33 (%)	p value
Physical activities			
Yes	42 (40.0)	6 (18.2)	0.037 [b] *
No	63 (60.0)	27 (81.8)	
Duration of physical activities			
No	63 (60.0)	27 (81.8)	0.059 [a]
≤ 2 days/week	28 (26.7)	3 (9.1)	
> 2 days/week	14 (13.3)	3 (9.1)	
Intensity of physical activities			
No	63 (60.0)	27 (81.8)	0.022 [a] *
< 1 Hour/day	11 (10.5)	4 (12.1)	
≥ 1 Hours/day	31 (29.5)	2 (6.1)	
Smoking			
Yes	62 (59.0)	19 (57.6)	0.881 [b]
No	43 (41.0)	14 (42.4)	
Duration of smoking			
No smoking	43 (41.0)	14 (42.4)	0.057 [a]
1 – 20 years	4 (3.8)	4 (12.1)	
21 – 40 years	37 (35.2)	5 (15.2)	
> 40 years	21 (20.0)	10 (30.3)	
Intensity of smoking			
No Smoking	43 (41.0)	14 (42.4)	0.737 [a]
1 – 2 Cigarette(s)/day	2 (1.9)	1 (3.0)	
3 – 5 Cigarettes/day	51 (48.6)	17 (51.5)	
≥ 6 Cigarettes/day	9 (8.6)	1 (3.0)	
Alcohol consumption			
Yes	32 (30.5)	9 (27.3)	0.894 [b]
No	73 (69.5)	24 (72.7)	

[a] Computed using Fisher's Exact Test; [b] Computed using Continuity Correction; * Statistically significant ($p<0.05$).

4.7 Association of Demographic, Clinical and Lifestyle Factors with TT Level

Ethnicity was the only demographic factor found not to be associated with TT ($p=0.758$). Meanwhile none of the clinical factors were significantly associated with TT. However, lifestyle factors such as physical activities, duration and intensity of physical activities, and duration of smoking were significantly associated with TT level. Tables 4.8, 4.9 and 4.10 respectively show the association of demographic, clinical and lifestyle factors with TT level after adjustment of confounding variable (age).

Table 4.8: Association of Demographic Factors with Total Testosterone Level (n = 138)

Demographic factors	Total Testosterone ≤ 9.55 nmol/L n=33 (%)	Total Testosterone > 9.55 nmol/L n=105 (%)	p Value
Ethnicity			
Malay	15 (45.5)	53 (50.4)	0.758 [a]
Chinese	11 (33.3)	28 (26.7)	
Indian	7 (21.2)	24 (22.9)	
Marital Status			
Married	28 (84.8)	105 (100.0)	0.001 [b] *
Others	5 (15.2)	0 (0.00)	
Occupation			
Government Employee	3 (9.1)	33 (31.4)	< 0.001 [b] *
Non-government employee	2 (6.1)	27 (25.7)	
Self Employed	5 (15.2)	16 (15.2)	
Pensioner	15 (45.5)	15 (14.3)	
Unemployed	8 (24.2)	14 (13.3)	
Academic Qualification			
Primary	7 (21.2)	4 (3.8)	0.002 [b] *
Secondary	25 (75.8)	86 (81.9)	
Tertiary	1 (3.0)	15 (14.3)	
Monthly Household Income			
Low	16 (48.5)	14 (13.3)	< 0.001 [b] *
Medium	14 (42.4)	51 (48.6)	
High	3 (9.1)	40 (38.1)	

[a] Computed using Pearson Chi Square; [b] Computed using Fisher's Exact Test; * Statistically significant ($p<0.05$)

Table 4.9: Association of Clinical Factors with Total Testosterone level (n = 138)

Clinical factors	Total Testosterone ≤ 9.55 nmol/L n=33 (%)	Total Testosterone > 9.55 nmol/L n=105 (%)	p Value
Type 2 Diabetes Mellitus			
Present	20 (60.6)	50 (47.6)	0.270 [b]
Absent	13 (39.4)	55 (52.4)	
Hypertension			
Present	17 (51.5)	68 (64.8)	0.246 [b]
Absent	16 (48.5)	37 (35.2)	
Hyperlipidemia			
Present	18 (54.5)	47 (44.8)	0.434 [b]
Absent	15 (45.5)	58 (55.2)	
Coronary Artery Disease			
Present	21 (63.6)	59 (56.2)	0.580 [b]
Absent	12 (36.4)	46 (43.8)	
Lower Urinary Tract Symptoms/ Benign Prostatic Hyperplasia			
Present	14 (42.4)	28 (26.7)	0.134 [b]
Absent	19 (57.6)	77 (73.8)	
Sildenafil 100mg			
Yes	3 (9.1)	31 (29.5)	0.080 [a]
No	30 (90.9)	74 (70.5)	
Sildenafil 50mg			
Yes	19 (57.6)	43 (41.0)	0.140 [b]
No	14 (42.4)	62 (59.0)	
Tadalafil 20mg			
Yes	3 (9.1)	19 (18.1)	0.282 [a]
No	30 (90.9)	86 (81.9)	
Vardenafil 10mg			
Yes	5 (15.2)	4 (3.8)	0.058 [a]
No	28 (84.8)	101 (96.2)	
Vardenafil 20mg			
Yes	3 (9.1)	8 (7.6)	0.724 [a]
No	30 (90.9)	97 (92.4)	

[a] Computed using Fisher's Exact Test; [b] Computed using Continuity Correction; * Statistically significant ($p<0.05$).

Table 4.10: Association of Lifestyle Factors with Total Testosterone Level (n = 138)

Lifestyle Factors	Total Testosterone ≤ 9.55 nmol/L n=33 (%)	Total Testosterone > 9.55 nmol/L n=105 (%)	p value
Physical activities			
Yes	2 (6.1)	46 (43.8)	< 0.001[a] *
No	31 (93.9)	59 (56.2)	
Duration of physical activities			
No	31 (93.9)	59 (56.2)	< 0.001[a] *
≤ 2 days/week	1 (3.0)	30 (28.6)	
> 2 days/week	1 (3.0)	16 (15.2)	
Intensity of physical activities			
No	31 (93.9)	59 (56.2)	< 0.001[a] *
< 1 Hour/day	1 (3.0)	14 (13.3)	
≥ 1 Hour/day	1 (3.0)	32 (30.5)	
Smoking			
Yes	21 (63.6)	60 (57.1)	0.647 [b]
No	12 (36.4)	45 (42.9)	
Duration of smoking			
No smoking	12 (36.4)	45 (42.9)	0.019 [a] *
1 – 20 years	3 (9.1)	5 (4.8)	
21 – 40 years	5 (15.2)	37 (35.2)	
> 40 years	13 (39.4)	18 (17.1)	
Intensity of smoking			
No Smoking	12 (36.4)	45 (42.9)	0.104 [a]
1 – 2 Cigarette(s)/day	0 (0.0)	3 (2.9)	
3 – 5 Cigarettes/day	21 (63.6)	47 (44.7)	
≥ 6 Cigarettes/day	0 (0.0)	10 (9.5)	
Alcohol consumption			
Yes	11 (33.3)	30 (28.6)	0.761[b]
No	22 (66.7)	75 (71.4)	

[a] Computed using Fisher's Exact Test; [b] Computed using Continuity Correction; * Statistically significant (p<0.05)

4.8 Association of Demographic, Clinical and Lifestyle Factors with ADMA Level

Association of demographic factors with ADMA (Table 4.11) shows ethnicity and marital status were not associated with ADMA level. None of the clinical factors were significantly associated with ADMA level. Meanwhile, physical activities and intensity of physical activities were the lifestyle factors which were significantly associated with ADMA level. Association of demographic, clinical and lifestyle factors with ADMA level were presented in Table 4.11, 4.12 and 4.13 respectively.

Table 4.11: Association of Demographic Factors with Asymmetric Dimethylarginine, level (n=138)

Demographic factors	Asymmetric dimethylarginine ≤ 0.665 µmol/L n=67 (%)	Asymmetric dimethylarginine > 0.665 µmol/L n=71 (%)	p Value
Ethnicity			
Malay	31 (46.3)	37 (52.1)	0.267 [a]
Chinese	17 (25.4)	22 (31.0)	
Indian	19 (28.4)	12 (16.9)	
Marital Status			
Married	67 (100.0)	66 (93.0)	0.059 [b]
Others	0 (0.0)	5 (7.0)	
Occupation			
Government Employee	21 (31.3)	15 (21.1)	0.006 [a] *
Non-government employee	20 (29.9)	9 (12.7)	
Self Employed	11 (16.4)	10 (14.1)	
Pensioner	9 (13.4)	21 (29.6)	
Unemployed	6 (9.0)	16 (22.5)	
Academic Qualification			
Primary	2 (3.0)	9 (12.7)	0.014 [b] *
Secondary	53 (79.1)	58 (81.7)	
Tertiary	12 (17.9)	4 (5.6)	
Monthly Household Income			
Low	5 (7.5)	25 (35.2)	<0.001[a]*
Medium	33 (49.3)	32 (45.1)	
High	29 (43.3)	14 (19.7)	

[a] Computed using Pearson Chi Square; [b] Computed using Fisher's Exact Test; * Statistically significant ($p<0.05$)

Table 4.12: Association of Clinical Factors with Asymmetric Dimethylarginine Level (n=138)

Clinical factors	Asymmetric dimethylarginine ≤ 0.665 µmol/L n=67 (%)	Asymmetric dimethylarginine > 0.665 µmol/L n=71 (%)	p Value
Type 2 Diabetes Mellitus			
Present	31 (46.3)	39 (54.9)	0.397 [b]
Absent	36 (53.7)	32 (45.1)	
Hypertension			
Present	45 (67.2)	40 (56.3)	0.258 [b]
Absent	22 (32.8)	31 (43.7)	
Hyperlipidemia			
Present	29 (43.3)	36 (50.7)	0.483 [b]
Absent	38 (56.7)	35 (49.3)	
Coronary Artery Disease			
Present	35 (52.2)	45 (63.4)	0.249 [b]
Absent	32 (47.8)	26 (36.6)	
Lower Urinary Tract Symptoms/ Benign prostatic hyperplasia			
Present	16 (23.9)	26 (36.6)	0.150 [b]
Absent	51 (76.1)	45 (63.4)	
Sildenafil 100mg			
Yes	20 (29.9)	14 (19.7)	0.237 [b]
No	47 (70.1)	57 (80.3)	
Sildenafil 50mg			
Yes	29 (43.3)	33 (46.5)	0.837 [b]
No	38 (56.7)	38 (53.5)	
Tadalafil 20mg			
Yes	9 (13.4)	13 (18.3)	0.583 [b]
No	58 (86.6)	58 (81.7)	
Vardenafil 10mg			
Yes	4 (6.0)	5 (7.0)	0.799 [a]
No	63 (94.0)	66 (93.0)	
Vardenafil 20mg			
Yes	5 (7.5)	6 (8.5)	0.830 [b]
No	62 (92.5)	65 (91.5)	

[a] Computed using Fisher's Exact Test; [b] Computed using Continuity Correction; * Statistically significant ($p<0.05$).

Table 4.13: Patients Lifestyle Factors Associated with Asymmetric Dimethylarginine Level (n = 138)

Lifestyle Factors	Asymmetric dimethylarginine ≤ 0.665 µmol/L n=67 (%)	Asymmetric dimethylarginine > 0.665 µmol/L n=71 (%)	p value
Physical activities			
Yes	30 (44.8)	18 (25.4)	0.027 [c] *
No	37 (55.2)	53 (74.6)	
Duration of physical activities			
No	37 (55.2)	53 (74.6)	0.055 [a]
≤ 2 days/week	19 (28.4)	12 (16.9)	
> 2 days/week	11 (16.4)	6 (8.5)	
Intensity of physical activities			
No	37 (55.2)	53 (74.6)	0.006 [a] *
< 1 Hour/day	6 (9.0)	9 (12.7)	
≥ 1 Hour/day	24 (35.8)	9 (12.7)	
Smoking			
Yes	39 (58.2)	42 (59.2)	0.910 [c]
No	28 (41.8)	29 (40.8)	
Duration of smoking			
No smoking	28 (41.8)	29 (40.8)	0.160 [b]
1 – 20 years	5 (7.5)	3 (4.2)	
21 – 40 years	24 (35.8)	18 (25.4)	
> 40 years	10 (14.9)	21 (29.6)	
Intensity of smoking			
No Smoking	28 (41.8)	29 (40.8)	0.189 [b]
1 – 2 Cigarette(s)/day	1 (1.5)	2 (2.8)	
3 – 5 Cigarettes/day	30 (44.8)	38 (53.5)	
≥ 6 Cigarettes/day	8 (11.9)	2 (2.8)	
Alcohol consumption			
Yes	21 (31.3)	20 (28.2)	0.825 [c]
No	46 (68.7)	51 (71.8)	

[a] Computed using Pearson Chi Square; [b] Computed using Fisher's Exact Test;
[c] Computed using Continuity Correction; *statistically significant ($p<0.05$)

4.9 Predictors of Severity of ED

Variables which gave significant result for both case & control (primary analysis) and severity group (secondary analysis) were subjected to this binary analysis. The Hosmer & Lemeshow test (X^2 = 6.592, p=0.581) for severity of ED shows the entered data was compatible with this binary logistic regression test. The Nagelkerke R^2 value was 0.693. The significant predictors for severity of ED in this study population were TT (p = 0.001) and occupation (p = 0.03). Based on the OR value, self-employed was ranked as first predictor which was 10.55 times most likely to increase severity of ED followed by pensioner (OR = 8.07) and non-government employee (OR = 1.16). Meanwhile, TT levels were 0.41 times more likely to worsening severity of ED in Malaysian population (Table 4.14).

Table 4.14: Predictors of Severity of ED

Predictors		OR	95% CI		p-value
			Low	High	
Occupation	Government				0.03*
	Non-government	1.16	0.05	26.26	0.04*
	Self employed	10.55	0.43	257.06	0.004*
	Pensioner	8.07	0.19	352.45	0.026*
	Unemployed	4.04	0.31	52.25	0.071
TT		0.41	0.25	0.69	0.001*
ADMA		320.75	0.77	133030.84	0.06
Academic	Primary				0.10
Qualification	Secondary	0.02	0.00019	1.18	0.06
	Tertiary	0.01	0.00031	0.68	0.07
Monthly Income	Low				0.21
	Medium	36.86	0.60	2269.33	0.09
	High	10.73	0.22	521.76	0.23
Physical activity	Yes				0.408
	No	1.57	0.16	15.45	0.701
Intensity of	No				0.82
Physical activity	< 1 Hour/day	1.34	0.12	15.30	0.82
	≥ 1 Hour/day	1.07	0.88	1.26	0.86

Binary logistics regression test was done to obtain the OR and p value;
*statistically significant ($p<0.05$)

4.10 Predictors of Total Testosterone (TT) Level

Significant variables from both primary and secondary association analysis were subjected to this binary analysis. The overall model was statistically significant. The Hosmer & Lemeshow test (X^2 = 10.724, p=0.218) shows the entered data was compatible with this binary logistic regression test meanwhile the Nagelkerke R^2 and Cox & Snell R Square values were 0.511 and 0.341 respectively. The only significant predictor which influenced TT level in Malaysian ED patients was occupation (p = 0.023). Specifically, pensioner (0.038) and unemployed (0.007) decreased the TT level by 0.08 and 0.04 times respectively. The binary regression result was presented in Table 4.15.

Table 4.15: Predictors of Total Testosterone Level

Predictors		OR	95% CI		p-value
			Low	High	
Occupation	Government				0.023*
	Non-government	0.50	0.06	4.56	0.542
	Self employed	0.46	0.03	6.62	0.571
	Pensioner	0.08	0.01	0.87	0.038*
	Unemployed	0.04	0.01	0.42	0.007*
Academic	Primary				0.108
Qualification	Secondary	0.06	0.01	1.71	0.099
	Tertiary	0.33	0.02	6.10	0.458
Monthly Income	Low				0.102
	Medium	0.08	0.01	0.84	0.335
	High	1.26	0.15	10.82	0.835
Physical activity	No				0.999
	Yes	7.03	0.25	195.13	0.250
Duration of	No				0.755
Physical activity	≤ 2 days/week	2.16	0.05	91.51	0.686
	> 2 days/week	2.65	0.95	50.51	0.686
Intensity of	No				0.932
Physical activity	< 1 Hour/day	0.87	0.04	20.13	0.932
	≥ 1 Hour/day	0.48	0.04	52.39	0.827

Table 4.15, continued

Predictors		OR	95% CI		p-value
			Low	High	
Duration of	No smoking				0.628
smoking	1 – 20 years	1.40	0.39	4.99	0.608
	21 – 40 years	0.24	0.01	4.13	0.324
	> 40 years	1.46	0.31	7.00	0.635

Binary logistics regression test was done to obtain the OR and p value;
*statistically significant ($p<0.05$)

4.11 Predictors of Asymmetric Dimethylarginine (ADMA) Level

Variables which gave significant result from primary and secondary analysis were chosen for this binary logistic regression test. The Hosmer & Lemeshow test value (X^2 = 3.152, p=0.924) for ADMA analysis shows the data was compatible with this binary logistic regression test meanwhile the Nagelkerke R^2 and Cox & Snell R Square values were 0.291 and 0.218 respectively. Intensity of physical activity (p = 0.018) and academic qualification (p = 0.047) were reported as the significant predictors which influenced ADMA level in Malaysian ED population. Spending less hours (inactive) for physical activities are 2.61 times most likely increasing ADMA level than active men (OR = 0.87). Meanwhile, men with secondary academic qualification are 4.51 times most likely to increase ADMA level than men with tertiary academic qualification level (OR = 1.88). The binary regression result was presented in Table 4.16.

Table 4.16: Predictors of Asymmetric Dimethylarginine (ADMA) level

Predictors		OR	95% CI		p-value
			Low	High	
Occupation	Government				0.109
	Non-government	5.40	0.45	64.15	0.181
	Self employed	1.89	0.16	22.73	0.615
	Pensioner	6.23	0.51	76.70	0.153
	Unemployed	7.15	0.64	80.36	0.111
Academic	Primary				0.047*
Qualification	Secondary	4.51	0.48	42.83	0.014*
	Tertiary	1.88	0.48	7.40	0.341
Monthly Income	Low				0.418
	Medium	20.99	1.87	235.25	0.190
	High	1.85	0.52	6.60	0.367
Physical activity	Yes				
	No	0.52	0.19	1.41	0.198
Intensity of	No				0.018*
Physical activity	< 1 Hour/day	2.61	0.65	10.48	0.008*
	≥ 1 Hour/day	0.87	0.18	4.21	0.060

Binary logistics regression test was done to obtain the OR and p value;
*statistically significant ($p<0.05$)

CHAPTER 5: DISCUSSION

5.1 Demographic Characteristics

Age of patients ranged from 40 to 82 years with the mean age of ED patients being 58.64 ± 9.24 years. This was similar to the data reported by Khoo (2008) with the mean age range of 58 ± 7 years. This proved that ED is more prevalent in older men. Nearly half of the patients were Malays, followed by Chinese and Indians. This proportion of race group is based on Malaysia's racial distribution. Similar results were also reported in a study by Mariappan et al. (2006) (Mariappan & Chong, 2006). However, another study did not agree with this finding. This study which was carried out by Khoo et al (2008) in the Selangor state of Malaysia had reported a higher percentage of Chinese patients as compared to Malay and Indian patients (Khoo et al., 2008). The variation in racial population was probably due to the difference in total population by racial population in the area of where these studies were conducted. Patients were predominantly married and this aspect was comparable with other related studies conducted in Malaysia (Huri et al., 2016; Khoo et al., 2008). However, studies conducted worldwide were not in agreement with the results obtained through studies held in Malaysia where the percentage of widowers were higher as compared to married men (Weinberg, Eisenberg, Patel, Chertow, & Leppert, 2013). This difference was probably due to clinical characteristics of the study population as the research was conducted on diabetic ED patients. From our results, it was found that majority of patients were government employees and pensioners, and this finding is in accordance to another study conducted in UMMC (Huri et al., 2016). However, that study has higher number of self-employed patients and this characteristic was not comparable with our study. In terms of academic qualification, our finding was in line with the study conducted by Fadzil (2010) whereby the number of patients with secondary education was much higher than that of with tertiary education (Fadzil et al., 2014).

Majority of patients were earning medium to high monthly income and this finding was reflected in many studies conducted in Malaysian context (Huri et al., 2016; Khoo et al., 2008; Momtaz et al., 2014). This was because all studies were conducted in urban area whereby most of the participants had professional and highly paid jobs.

5.2 Clinical Characteristics

This study revealed that majority of ED patients were suffering from multiple comorbidities. The most common diseases among participants were hypertension, contributing to the highest percentage of disease, followed by CAD. This finding was supported by a review article which explained the association between these two diseases on how they prevent the dilation of arteries around penile region to initiate ED (Javaroni & Neves, 2012). Several research studies also revealed that ED was prevalent in hypertension patients in Malaysia (Fadzil et al., 2014) and globally (Cordero et al., 2010). The pathophysiology linking hypertension and ED was well described by Viigimaa *et al.* (2011) (Viigimaa et al., 2011). Meanwhile, many evidence-based studies or reviews had been published to support the association between CAD and ED (Gandaglia et al., 2014; G Jackson et al., 2010). Approximately half of the ED patients in this study were suffering from T2DM. This finding was consistent with previous studies which proved that the impairment of nitric oxide synthase as a risk factor of ED in diabetic patients (Angulo et al., 2010; Giugliano et al., 2010; Thorve et al., 2011). Hyperlipidaemia was also reported to be a common disease in ED patients and this result was in line with an animal study conducted in University of California (Qiu et al., 2011). This study concluded that the structural changes such as reduction of nNOS-positives nerves, endothelium and an increase in cavernous smooth muscle in penis as reasons for development of ED in hyperlipidaemic patients. Finally, BPH associated LUTS was the least common comorbidity reported among the patients in our study and was documented as one of the factors leading to risk of having ED (Dutkiewicz et al.,

2012). Our result showed that TT and ADMA were statistically significantly associated with ED ($p < 0.001$). This implied that TT level recorded in ED patients was lower as compared to the control group. On the other hand, a higher mean ADMA levels were found in ED patients. Previously the published studies have supported this finding, in which the severity of ED also increased as the TT level decreased (Brooke et al., 2014) and ADMA level increased (Aktoz et al., 2010; Paroni et al., 2012).

5.3 Lifestyle Characteristics

Participants of this study were engaged in physical activities. It was reported that 48 (34.8%) of ED patients were engaged in physical activities, out of which, 31 (22.5%) had at least spent 2 days per week for physical activities. In terms of the intensity of physical activities, 15 (10.9%) of the ED patients spent minimum 1 hour per day for some physical activities whereas 33 (23.9%) spent more than 2 hours in physical activities. Two-thirds of the ED patients were reported to have had no extreme physical activities. On the other hand, nearly 60% of the control group participants were actively engaged in physical activities whereby 58 (42.0%) of them spent more than 2 days per week and 30.4% ($n = 42$) of them spent more than 1 hour per day in doing some physical activities. This result was consistent with some previous studies which showed that there was an improvement in IIEF scores among the physically active group as compared to the control group and these studies clearly explained the importance of physical activities in restoring erectile function (Lamina et al., 2009; Maio, Saraeb, & Marchiori, 2010; Wing et al., 2010). Out of 138 cases, 81 (58.7%) participants were smokers, and 57 (41.3%) were non-smokers. Meanwhile about 58.7% from the control group participants were categorized as non-smokers. This result is indicating that ED was more prevalent among smokers and this finding is in accordance with (Cao et al., 2013) whereby it was reported that smoking has significant correlation with ED and that the duration of smoking is associated with higher risk of having ED (Wu et al., 2012b).

Surprisingly, the number of non-alcoholics having ED is much greater than that of the alcoholics with 70.3% of case group and 79.0% of control group being non-alcoholics. This was because majority of patients in our study were from Malay ethnic who were forbidden from drinking alcohol.

5.4 Demographic Factors Associated with Severity of ED

Ethnicity is insignificantly associated with severity of ED in this study ($p = 0.951$). However, the mean IIEF scores were reported to be higher among the Malay and Indian communities as compared to the Chinese community. However, this finding was in contrast with the study carried out by Tan (2007), which reported that severe ED was highly associated among the Malays and Indians ($p < 0.001$) (Tan et al., 2007). The main reason for this discrepancy was due to the variation in health-seeking behaviours by race in treating ED (DeLamater & Karraker, 2009). Social, cultural and religious teachings have impact on an individual's view towards sexual activity (Ravanipour et al., 2013). Study by Guan (2004) cited Chinese society considers sexual intercourse is solely for reproduction purpose (Guan, 2004), subsequently the medical requirements for sexual dysfunction have likely been suppressed among this ethnic group. In addition, diverse genetic and distinctive hereditary factors among different ethnic groups might influence the association between ethnicity and the severity of ED. The difference in the DNA sequence of some genes which contributes to the risk for developing ED varies among ethnics. However, further studies need to be carried in understanding how genes can affect the severity of ED.

Our study revealed a significant association between marital status and the severity of ED ($p = 0.001$). In this study, the mean IIEF scores were higher among married men than the other groups (unmarried, divorced and widowed). This finding was in accordance with other studies conducted previously (Khoo et al., 2008; Millett et al., 2006). However, our result contradicts the data obtained by Oyekanmi, where marital

status was reported to be insignificant with the severity of ED ($X2 = 5.332$, $df = 4$, ($p = 0.255$) (Oyekanmi et al., 2012). Another study conducted by Laumann reported that non-married men are 1.73 times more likely to have severe ED than the widowers or divorcees (OR = 1.61) (Laumann, Paik, & Rosen, 1999). Married life gives men a chance to obtain "feedback" from their respective life partners about their deficiency in sexual performance especially ED. This could alert them of the current sexual dysfunction which generally go unnoticed by the patients and help them to take initiative steps to seek better treatment for the problems (Oyekanmi et al., 2012). However, single men are probably unaware of their sexual dysfunction because of no partner to alert them and are less likely to seek treatment. This possibly explained the significant difference between marital status and severity of ED. In addition, most of the men in this study are in the middle age range with a mean age of 58.64 ± 9.24 year, thus both the men and their life partners will have a tendency to be more understanding with one another's physical condition and provide additional importance for a better health (Huri et al., 2016).

Our study showed that occupation is significantly associated with the severity of ED and this finding is supported by a few studies conducted previously (AlMogbel, 2014; Aytaç et al., 2000). This study envisions that patients' ED management is prone to be affected by occupation status. In this study, IIEF scores were reported to be higher among patients with better employment status with less workload and work stress. This explains that men with stable employment, who are also generously compensated have a greater sense of control over their wellbeing (Momtaz et al., 2014). Besides that, ED is severe among patients with unstable employment as this could be due to financial constraints in treating ED. The costs associated with ED treatment can be a financial burden. Therefore, the ED treatment is unaffordable for some patients who have unsecured job coupled with high living expenses (Hackett, 2005). This situation

explains the huge difference in severity of ED among patients in different categories of occupation.

Our study revealed a significant association between academic qualification and severity of ED ($p = 0.006$). This finding was similar to some previous studies (Johannes et al., 2000; Laumann et al., 1999; Mirone et al., 2004). Severe ED was found to be lower in educated men. This could be due to the probability that highly educated men are generally well paid, have better access to medical treatment and therefore lead a healthy lifestyle. Besides that, men with better academic qualification are more likely to have better awareness on their health and thus give a higher priority for health and proper treatment for illness (Adler & Newman, 2002). Furthermore, they are also reported to have lower risk of comorbidities that are strongly associated with ED, such as diabetes and cardiovascular diseases (Johannes et al., 2000). Better health is strongly associated with improved sexual function (Adler & Newman, 2002). This clarifies why ED is less severe among educated people.

Existing studies reported that income levels were significantly associated with severity of ED (Almogbel, 2014; Martin et al., 2014; Momtaz et al., 2014). This finding is in accordance with results obtained in our study where monthly income is significantly associated with severity of ED ($p < 0.001$). Moreover, the mean IIEF scores also increased gradually with increasing monthly income. A study by Wong et al. (2006) reported that lower income is significantly associated with risk of having lower level of male sex hormone (Wong, Chan, Hong, & Woo, 2006). Moreover, Laumann reported that a two fold increment in prevalence of ED was recorded with decreasing household income (Laumann et al., 1999). This explains that low monthly income leads to poorer access to healthcare centres which severely influences men's health. Furthermore, men with lower income always give priority for their essential needs in

life instead of treatments which eventually results in their ED being untreated (Hackett, 2005).

Despite the fact that this study was conducted in an urban setting with majority of participants having at least secondary academic qualification with medium to high monthly income, ED can still be a problem among these patients. The prospective reasons for this finding might be that most patients are middle-aged with a mean age of 58.64 ± 9.24 years old and have had gradual decline in their physiology function as they age. This study revealed that patients' ED managements were impacted by demographic factors and clinicians should persistently educate patients regardless of their demographic, education or financial background.

5.5 Clinical Factors Associated with Severity of ED

It was found that type of comorbidities were not significantly associated with severity of ED. Moderate to severe ED was reported to be higher in patients with T2DM. Besides that, 57.6% of hypertension patients had severe ED. The same result was obtained for hyperlipidemia patients too whereby 57.6 % of them were suffering from moderate to severe ED. Meanwhile, 66.7% of ED patients with CAD were having severe ED. In contrast, severe ED was less prevalent in LUTS/BPH patients.

From this study, it can be observed that TT ($p < 0.001$) was significantly associated with severity of ED. Furthermore, binary regression analysis indicated low TT level as a significant predictor which was more likely to cause severe ED, (OR=0.41). High level of TT might not heavily affect erectile function, however low TT level can have significant impact on the severity level of ED (Mikhail, 2006). This was proven in a few studies which explored significant effect of low TT level on the severity of ED (Brooke et al., 2014; Liao et al., 2012; Martínez et al., 2006). TT acts as a vasodilator for the penile arterioles. The vasodilation of the arteries around the corpora cavernosa allows

more blood to flow in to help maintain the erection. Additionally, TT activates the Nitric Oxide synthase (NOS) and increases the expression of NO and phosphodiesterase type 5, which are essential enzymes for erection (Blute et al., 2008). Besides that, testosterone restores structural, biochemical and physiological changes and induces new DNA synthesis in the smooth muscle cells and blood vessels. In addition, testosterone also contributes to venous occlusion and helps to maintain erection (Shabsigh, 2005).

Similar to TT, ADMA is also found to be the significant clinical factor associated with the severity of ED ($p < 0.001$). This finding was in agreement with few prior studies (Aktoz et al., 2010; Paroni et al., 2012). ADMA is an extract from the methylation of arginine from protein residues that are catalysed by protein enzymes called arginine N-methyltransferases. This reaction transfers a methyl group from S-adenosyl-L-methionine to guanidine nitrogen of arginine residues and forms a methylated arginine derivative protein that contains ADMA. The hydrolysis of the methylated proteins releases ADMA (Landim, Casella Filho, & Chagas, 2009). The synthesis of ADMA is altered by metabolism of DDAH. Inhibition of DDAH activity causes accumulation of ADMA (Sibal et al., 2010). Since ADMA is an endogenous inhibitor of NO synthase, it can degenerate the synthesis of NO which inhibits the relaxation of smooth muscle in penile tissues and leads to severe ED (Kwiecien et al., 2012). Erectile function is much dependent on NO for vasodilation of corpus cavernosum for a rigid penile erection. Elevated ADMA causes NO deficiency and degeneration of the cGMP level; which will eventually lead to severe ED (Aktoz et al., 2010; Gareri et al., 2014). Association of ADMA with ED is higher with the presence of other factors such as endothelial dysfunction. When comparing to other cells in the body, the level of ADMA is 10-fold higher in endothelial cells (Davies & Melman, 2008). Thus, any damage to endothelial cells will result in the possibility of endothelial

dysfunction and an increase in the ADMA serum level. These effects, in turn, increase the severity of ED (Javaroni & Neves, 2012; Sibal et al., 2010).

5.6 Lifestyle Factors Associated with Severity of ED

In this study, the association of physical activity and severity of ED is found to be significant ($p = 0.037$). Besides that, the intensity of physical activity ($p = 0.022$) is also found to be significant with the severity of ED. These findings are in accordance with previous reports (Bacon et al., 2006; Esposito et al., 2009; Hsiao et al., 2012; Kałka et al., 2013; Malavige et al., 2015; Selvin et al., 2007). Improvement in physical activity decreases the risk of developing severe ED. In addition, a recent study conducted on ED patients with IHD reported that increasing the intensity of physical activities positively reduces the risk of advancing the severity of ED (Kałka et al., 2013). The lifestyle changes (intensity and duration of physical activity) suggest that, increasing exercise level in terms of time and days might be effective non-pharmacological treatments for ED patients. Changes in lifestyle may play a role in healing or treating ED. Physical activities improve erectile function by various mechanisms including enhancing endothelial function (Meldrum et al., 2012), increasing endothelially-derived nitric oxide (NO) (Meldrum, Burnett, Dorey, Esposito, & Ignarro, 2014) and diminishing oxidative stress (Esposito et al., 2009). Consistent physical activities may stimulate NO synthesis in ED patients. NO enhances relaxation of smooth muscle and allows blood flow into arteries around penile region, which are essential aspects for erection (Clavijo, Miner, & Rajfer, 2014). Persistent physical activities could alter the biochemical, neural and hormonal changes and result in blood vessel relaxation. In addition, physical activities help the synthesis of specific chemical reaction such as NO and lactic acid, and changes nerve activities and hormones which enhance dilation of blood vessels (Lamina et al., 2011). Besides that, physical activities also help to diminish risk factors

of ED such as insulin resistance, inflammatory markers and enhance vascular function (La Vignera et al., 2013).

Smoking history, duration and intensity of smoking appeared to be insignificant with severity of ED in this study. This result was in contrast with a study carried out by Pourmand where intensity of smoking cigarette was found to have a strong correlation with the severity of ED (Pourmand et al., 2004). Recently, Chan (2010) reported that discontinuing smoking decreases the risk of developing severe ED and significantly enhances erectile function (Chan et al., 2010). This explains the need for an effort to encourage people to stop smoking and this should be targeted at teenagers, in order to raise the awareness on the effect of smoking on erectile function. Although the effect of smoking on pathophysiology of erectile dysfunction is not well known but a few studies have reported that smoking can damage the small blood vessels around corpora cavernosa that are required for initiating and maintaining an erection (Natali, Mondaini, Lombardi, Del Popolo, & Rizzo, 2005). Besides that, NOS expression by non-adrenergic and non-cholinergic neurons was severely affected by cigarette smoke. Furthermore, components in cigarettes and cigarette smoke were responsible for the endothelial cells injuries and vascular damages which eventually weaken the endothelium dependent vasodilation (Tostes et al., 2008). Other than that, superoxide anions were found to be elevated among smokers. This superoxide anion drastically decreases bioavailability of NO and impairs erectile function (Orosz et al., 2007; Peluffo, Calcerrada, Piacenza, Pizzano, & Radi, 2009). Furthermore, reactive oxygen species (ROS) and superoxide anion (component of cigarette smoke) could cause oxidative damage to endothelial cells and eventually lead to severe ED (Mazzone et al., 2010).

5.7 Demographic Factors Associated with Total Testosterone (TT) Level

In this present study, ethnic group ($p = 0.758$) was the only demographic factor that was insignificantly associated with TT levels. Moreover, it was found that there was no significant difference in TT level between men from different ethnic subgroups (Malay, Chinese and Indian).

Study has revealed that marital status was significantly associated with TT levels ($p = 0.001$). Low TT level was recorded in widowers and this was supported by a prospective cohort study conducted on Massachusetts men where loss of spouse was significantly associated with decreased testosterone level (Travison, Araujo, Kupelian, O'Donnell, & McKinlay, 2007). TT was also found to be higher in married men as compared to men from other marital categories (non-married, widower and divorcee). This was because most participants in this study were married. However, this result was inconsistent with some previous studies' findings. A study by Farrelly (2015) revealed that men who were married or in a long term relationships had low testosterone level as compared to their counterparts (Farrelly, Owens, Elliott, Walden, & Wetherell, 2015). According to the author, men with low TT level are probably better guardians and look out for consistent relationship as compared to men with higher TT level who prefer to stay single or tend to end their relationship to look for a new companion (Farrelly et al., 2015). In accordance with this, married men are reported to have low TT levels as compared to unmarried/single men as they are more focused on a quality relationship, commitments and responsibilities as fathers (Edelstein, van Anders, Chopik, Goldey, & Wardecker, 2014; Gray, Kahlenberg, Barrett, Lipson, & Ellison, 2002). On the other hand, TT is also associated with aggression, mate-seeking behaviours and competition (Norman, Moreau, Welker, & Carré, 2015) and these traits tend to be higher in single men. These findings provide new evidences that men's relationship quality decreases as TT level increases. Moreover, the association between marital status and TT level are

mutual and influencing each other. Future studies should be conducted to examine the influence of marital status on the level of TT.

Apart from that, this study also examines the relationship between TT level and employment status. Occupation or employment status was reported to have a significant impact on the TT level (Greene et al., 2014, Geniole et al., 2016) Men with better employment status have higher TT level as compared to unemployed men and pensioners. This result was in accordance with multiple studies that had been conducted earlier, studying the significant impact of occupation on TT level. Men who have work stress are likely to have lower TT level. On the contrary, higher TT level helps in lowering stress and in giving more strength and inspiration to perform better at work (Greene, Han, Martin, Zhang, & Wittert, 2014). It is a known fact that stress may have physiological effects on body and cause infertility by decreasing TT level. Besides that, high stress and low TT level result in common physical symptoms including erectile dysfunction. There are a few studies evidencing that the stress hormone cortisol and TT level work against each other and a high stress level is strongly associated with low TT level. A meta-analysis study reported that cortisol level may have a significant effect on TT level (Geniole, Bird, Ruddick, & Carré, 2016). The notable psychological association between TT level and stress is not well known. It is most likely that the chemicals secreted in response to stress may regulate the TT generation and control its level.

Besides occupation, a significant association was reported between academic qualification and TT levels in this present study ($p = 0.002$). This result was consistent with a previous study which indicated that TT level was recorded to be higher in university graduates as compared to non-university graduates (Al Hayek et al., 2013). When compared to less educated men, the better educated men were much more aware on their health issues. Furthermore, they are ready to consult doctors for treatment

(Momtaz et al., 2014). Highly educated men are aware on factors which influencing lower TT level and take initiatives to prevent it. They were willing to get health care treatment to minimize these factors and lead a healthy life. On the other hand, men with low academic qualification probably have to work harder to survive. They might get tired and their body have insufficient rest, which could increase their stress level. Stress was significantly associated with low TT as explained previously (Greene et al., 2014). This could be the reason why TT levels vary with academic qualification.

This study also proved a significant association between monthly income and TT levels. Studies have been reported that low TT level was more prevalent in men who have low income level and financial standing was seen as a risk factor influencing men's TT levels. This finding was in accordance with a study by Al Hayek (2013), where income was reported as a risk factor for low serum TT levels ($p < 0.001$) (Al Hayek et al., 2013). A significant association was found between TT levels and socioeconomic position in a study conducted in British men. The study theorized that low TT level was significantly associated with low income and poor education qualification. The research concentrated on the differences in health level between rich and poor individuals and the report indicated that a child raised in a privileged environment was expected to live 8.5 years longer than the counterpart who comes from a poorer background (Bann et al., 2015). The same study also reported that individuals with lower income were more susceptible to develop diseases and disorders like depression, obesity, osteoporosis and muscle loss due to lower level of TT (Bann et al., 2015). Underprivileged men have lower chances to live healthy lifestyle as they lack access to quality food and proper exercise equipment which may be expensive. All these contribute to a more stressful lifestyle. It can be inferred that poorer individuals were "biologically different" from rich people and these could explain the differences in health level among them, especially on their TT level.

5.8 Clinical Factors Associated with Total Testosterone (TT) Level

In this study, no significant association was found between clinical factors and TT level. This shows that comorbidities such as T2DM, hypertension, hyperlipidemia, CAD and LUTS/BPH have no effects on TT level in ED patients. Our findings were totally in contrast with some of the previous studies where TT level was reported to be significantly low in Pomerania men with T2DM (Schipf et al., 2011), hypertension (Torkler et al., 2011), CAD (Hu et al., 2011) and in patients with BPH problem (Mazurek, Stępień, & Klukowski, 2004). Nevertheless, all these studies were conducted on non-ED population. Most of the studies didn't suggest that low TT causes these conditions. In fact, the link is both ways. Those with low TT have higher risk of developing of T2DM, Hypertension and CAD as well as men with these medical problems would develop low TT. According to a book on reversing diabetes published by Colbert (2012), researchers have identified a general link between low TT and type of comorbidities. A study consisting of 2100 men aged above 45 years old reported that the odds of having low TT level were 2.4 times higher in obese men. Besides, low TT levels were 2.1 times and 1.8 times higher in men with diabetes and hypertension respectively (Colbert, 2012). Low TT levels were generally associated with insulin resistance (Grossmann et al., 2008). Patients with insulin resistance have sufficient insulin production however the body does not use it properly. As a result, glucose levels become elevated in the blood rather than being absorbed by cells. This insulin resistance can lead to T2DM and increases the risk of chronic health problems including hypertension (Zhou, Wang, & Yu, 2014) and heart diseases such as CAD and atherosclerosis (Malkin et al., 2010; Pitteloud et al., 2005). Apart from comorbidities, PDE-5 inhibitors (sildenafil, tadalafil and vardenafil) also showed no influence on TT level. However, this finding was inconsistent with the study conducted by Carosa (2004) which reported an increase in TT level after PDE-5 inhibitors treatment (Carosa

et al., 2004). The variables were not significant probably due to the smaller sample size of this study.

5.9 Lifestyle Factors Associated with Total Testosterone (TT) Level

Among all the lifestyle factors, the smoking history, intensity of smoking and alcohol consumption were statistically not significant with TT level.

It was not surprising that a significant association was found between physical activity and TT level in this study. This finding was in line with the studies by Dandona & Rosenberg (2010) and Devi et al. (2014) who also found similar results that the intensity and duration of physical activities were found to be significant with TT levels (Ari et al., 2004; Khorshidi, Azizbeigi, & Abedi, 2014). Previous studies have reported high TT levels in physically active men as compared to their counterparts. A study conducted on Tsimane men demonstrated a significant rise in TT level after they spent about one hour for extreme physical activities (Trumble et al., 2013). Our understanding on hormonal response to exercise has increased with the results from this study. Our body's endocrine (hormonal) systems appeared to be effected in response to physical exercise (Ball, 2015). Endocrine system manipulates various physiological functions of our body. Studies have shown that exercise could help our endocrine system to secrete hormones more efficiently. This research has shown that increasing physical activities in terms of intensity and duration enhance our endocrine system, stimulate the release of luteinizing hormone and trigger TT production (Hackney, Moore, & Brownlee, 2005). The mechanism of physical activities that increase TT level is complex and had not been widely explored. Consistent physical activities alone probably will not increase TT level but appropriate diet along with healthy life style could impose stronger impact on TT production in men.

Duration of smoking is significantly associated with TT level ($p = 0.019$). It was found that men who smoke have higher levels of TT in their blood serum as compared

to non-smokers. This finding was in line with Katherine et al (2001) and Wang et al (2013), who reported that the level of TT was higher in smokers than in non-smokers. The findings on the effects of smoking on TT level in men were not consistent. Evidences reveal that smokers metabolize more TT and their livers dispose TT more effectively (Olayaki et al., 2008). In contrast, another study reported that smoking decreases zinc in our body which is a vital mineral for TT production (Al-Azzawy, 2011). Hypothetically, this would imply that smoking alongside with poor diet could lead to zinc deficiency and consequently lower the TT level. The relationship between TT and tobacco is complex, but tobacco is not the main reason behind low level of TT level. Based on a few studies, the presence of high nicotine level is believed to be the cause for lower TT level among smokers (Carvalho, Pissolato, Candido, Liberti, & Cagnon, 2012; Oyeyipo, Raji, Emikpe, & Bolarinwa, 2010). However, the exact pathology is debatable. This study proposes that smoking may or may not lower our TT level and it relies on the amount of nicotine inhaled by smokers.

5.10 Demographic Factors Associated with Asymmetric Dimethylarginine (ADMA) Level

Out of all the demographic factors, ethnic group ($p = 0.267$) and marital status ($p = 0.059$) were found to be non-significantly associated with ADMA levels. On the other hand, occupation, academic qualification and monthly household income were reported to be significant with ADMA levels. To the best of the authors' knowledge, this is the first study to examine the association between demographic factors and ADMA level in an ED population.

This study found that occupation was significantly associated with ADMA levels. More specifically, ADMA level was found to be higher in population with unstable jobs. In today's world, educated individuals who are involved with jobs that are not physically demanding are believed to have lower level of stress. On the other hand,

people having unstable jobs are more vulnerable to high stress level. Study evidenced that ADMA level is significantly elevated in people with unstable jobs, stress and depression in comparison with their healthier counterparts (Baranyi et al., 2015; Curgunlu et al., 2005). Jobs that are physically demanding have higher chance of increasing oxidative stress that could lead to accumulation of ADMA level. ROS which is responsible for oxidative stress level was found to be elevated in workers standing for a long time (Flore et al., 2004; Schieber & Chandel, 2014). The activity of DDAH was weakened by this oxidative stress allowing, ADMA to accumulate (Sibal et al., 2010). Therefore, this study revealed that different employment status along with stress and depression could play a major role in manipulating ADMA level in ED patients.

Academic qualification was found to be significant with ADMA level in this study. The link between education and ADMA level is complex and is tied closely to income. Although no studies were established on the association of academic qualification with ADMA level yet, there were few theories and hypotheses which can illustrate how education level can be associated with ADMA level in term of patients' health. Education can increase an individual's knowledge levels and sense of control over their life, which in turn would encourage them to embrace a healthier lifestyle. Educated men value their health highly. They constantly look for the guidance of a physician or an experienced health care provider about their health and these men are ready to communicate effectively with them**.** However, less educated people might be unaware of their health due to lack of knowledge on diseases and its symptoms, which compromises on their health. In addition, poor quality of doctor-patient communication contributes to the high percentage of health issue among this group (Verlinde, De Laender, De Maesschalck, Deveugele, & Willems, 2012). Low morbidity rate was reported among educated men and they are less likely to have poor health. In contrast, the opposite result was observed for less educated men (Sallis & Carlson, 2015;

Zimmerman & Woolf, 2014). Besides that, a few studies suggest that educated people have healthier behaviours. Those with higher education level were less likely to smoke, drink heavily, use illegal drugs or be overweight (Cutler & Lleras-Muney, 2010). In this study, the knowledge on chemical nature of biomarkers (ADMA) is essential as it will help them to predict how it is produced and the mechanism of action in our body. This knowledge will help an individual to be aware of the biomarker level changes and to seek medical help in order to overcome the problem.

As per author's knowledge, no studies had been reported so far on the association of income with ADMA level in ED population. In this study, monthly income was found to be significantly associated with ADMA levels. Although there are no studies that had been conducted previously, the theories of socioeconomic on health state could justify this association. Various studies have proposed that income is one of the fundamental reasons for healthy behaviours of an individual (Cutler & Lleras-Muney, 2010; Dubay & Lebrun, 2012; Pampel, Krueger, & Denney, 2010; Pilić & Džakula, 2013). An individual's health was greatly affected by their financial status. Individuals who were economically stable have a greater access to healthcare. Men who earn higher income were most likely to have the ability to pay for the services from healthcare centres whereby the costs are most likely covered by healthcare insurance company. Besides that, they have better access to healthy food and lifestyle. On the other hand, individuals who are financially unstable are more vulnerable during economically difficult situations and this would lead them to neglect their health and medical needs. Subsequently, lower income becomes an obstacle to obtain good healthcare services. This was supported by a study which reported that patients were not able to consult physicians due to unaffordable medical cost (System, Control, & Prevention, 2011). Individuals with low income are most likely living in environments with low access to good medical care and healthy food. Healthy food is essential for good health because

unhealthy dietary patterns were related to various acute and chronic health problems (Zimmerman & Woolf, 2014) which could influence biomarker level in body, especially the ADMA level. Thus, the above justifications explain that income or socioeconomic status have an indirect association with ADMA level in the ED population.

5.11 Clinical Factors Associated with Asymmetric Dimethylarginine (ADMA) Level

No significant association was found between T2DM and ADMA level. Approximately, 55% of T2DM patients were reported to have higher ADMA levels. A study by Malecki et al. (2007) found that ADMA level in diabetic patients was related to the bioavailability of NO which is essential for diabetes vascular complications (Malecki et al., 2007). The same results were obtained from previous studies whereby ADMA level was higher in patients with hypertension (Perticone et al., 2010) and CAD (Xuan et al., 2015). However, no specific study was found on association of ADMA levels with hyperlipidemia, BPH and PDE5 inhibitors, and this study could provide the required ground information for future research.

5.12 Lifestyle Factors Associated with Asymmetric Dimethylarginine (ADMA) Level

Physical activity and intensity of physical activity were identified as significant factors associated with ADMA level. Meanwhile, the duration of physical activity was found to be insignificant with ADMA level in ED patients. These findings were quite similar to the previous studies which reported significant association between physical activities and ADMA level. However, those studies were not conducted on ED population. In this study, ADMA level was found to be lower in men who were physically active. In agreement with our study, exercise was reported decrease plasma ADMA level in a study conducted by Mittermayer et al. (2005). Another study showed that marathon runners had decreased plasma ADMA level after exercising

(Papadopoulou et al. 2012). Recently, Kevin et al. (2011) showed that after a moderate to intense exercise, ADMA level decreased in patients from the initial level before they began the exercise (0.42 ± 0.02 and 0.43 ± 0.03 micromol/l). The ADMA level started to increase again after they discontinued their exercise (Kevin, 2011). A decrease in ADMA level was even recorded in an animal study after the rats were exposed to exercise training, although the result was statistically insignificant (Esfahani et al., 2013). These studies concluded that ADMA level can be lowered by consistent physical activities. The mechanism to decrease ADMA level associated with physical exercise is crucial. ADMA is produced in substantial amount through methylation of protein-bound L-arginine by protein methyltransferase (PRMT) enzyme and the decomposition of these ADMA by DDAH activity appears to determine the ADMA level in our body (Teplan et al., 2014). Intensive physical activities may affect antioxidant level through increased expression of the superoxide dismutase. The DDAH activity is reduced by the increased concentration of oxidants level and oxidative stress. Hence, the enhancement of antioxidant status through regular exercise might be related to the decrease in ADMA level in physically active individuals (Mittermayer et al., 2005; Teplan et al., 2014). Besides that, constant physical activity can initiate the endothelial NO-synthase (eNOS) expression through increased endothelial shear stress and arterial blood flow. As we discussed earlier in literature review, ADMA inhibits NO synthesis. However, increasing intensity of physical exercise elevate NO bioavailability. This explains that ADMA level is suppressed upon significant improvement in physical activities which resulted in increased concentration level of NO (Schlager et al., 2011). Furthermore, ADMA could trigger the development of atherosclerosis by inhibiting NO production from amino acid l-arginine. Hence, decreased ADMA levels may enhance endothelial function in ED patients who were physically active (Böger, 2006). Since high ADMA level was related to ED (Paroni et al., 2012), our findings demonstrate that physical

activity does not only decrease ADMA level but could also contribute to improvement in erectile function.

Other lifestyle factors such as smoking history (intensity and duration of smoking) and alcohol consumption were insignificantly associated with ADMA level in this study. However, ADMA level has been reported to be higher in smokers as compared to non-smokers (Schnabel et al., 2005). This could be the first study investigating the association of ADMA level with smoking in Malaysian ED patients. All previous studies associating ADMA with smoking were involving non-ED population. In this study, we observed higher ADMA level in smokers as compared to their non-smoking counterparts. Even more interesting is that ADMA level is higher in men who were smoking for more than 20 years and this fact suggests that duration of smoking and cigarette smoke may enhance metabolism of ADMA. Smoking cigarette frequently may increase ADMA concentration level and contribute to severe ED and other multiple disorders. Meanwhile, alcoholics were reported to have lower ADMA concentration as compared to non-alcoholics who recorded higher ADMA level. This could be due to the limited number of patients who were alcoholics in this study. So far, the effect of smoking and alcohol consumption on ADMA had been discussed quite inconsistently. The association of smoking and alcohol consumption with ADMA level cannot be clearly concluded and should be investigated further.

5.13 Predictors of Severity of ED

Occupation ($p = 0.03$) and TT level ($p = 0.001$) were significant predictors influencing the severity level of ED in Malaysian population. Particularly, self-employed men were 10.55 times more likely to have severe ED as compared to government employees. Besides that, pensioners (OR = 8.07) and non-government employees (OR = 1.16) were also more prone to have severe ED than government employees.

High workload and unemployment for a longer period of time can impact our health as well as emotional well-being. Stress eventually affects erection and impedes men's sexual life. Approaching a therapist or a doctor is the best solution to alleviate severe ED. Besides that, discussing the problem with partners by expressing our feeling and expectations could help affected men to feel better. A significant negative impact on sexual health was reported in a psychological study inspecting the association between sexual dysfunction and occupational stress. The study revealed that working longer hours and stress in accomplishing a work within a targeted time eventually affect sexual performance. The study showed that men with a higher workload are 1.8 times more likely to have issues in sexual relationship than men with less work strain (Štulhofer, Træen, & Carvalheira, 2013).

TT level was another significant predictor whereby it could influence the severity of ED in Malaysian patients by 0.41 times although it showed no higher impact. Investigation on TT level should be done in men who were having symptoms of ED. Significant results were obtained when we analysed the mean TT level with case and control group (without ED) at first, and then with ED severity sub-groups. This finding emphasized that there was a significant association between TT level and the severity of ED. Undeniably, testosterone therapy can be considered as a recommended and effective treatment for men with severe ED.

5.14 Predictors of Total Testosterone (TT)

The effect of demographic, clinical and lifestyle factors cause significant changes on binary regression model of TT level in ED patients. After all the significant variables of demographic, clinical and lifestyle factors are included into binary regression model, occupation (p = 0.023) remained as significant predictor. Findings revealed that pensioners (OR = 0.08, 95% CI = 0.01-0.87, p = 0.038) and unemployed men (OR = 0.04, 95% CI = 0.01-0.42, p = 0.07) were most likely to have low TT level as compared to government employees. Pensioners and unemployed men who were physically inactive compared to working men which eventually decreasing TT levels among them. Besides, pensioners who were reported have low testosterone level is simply due to the decline of normal aging and also due to multiples diseases (hypogonadism).

To the best of the author's knowledge, very few studies have been made on predictors of demographic factors on TT level in ED patients. However, some association studies have been conducted based on low TT level with occupation. Zitzman (2001) has reviewed a study and found that lower social status is significantly associated with lower TT level. Besides that, increasing stress level due to heavy workload could affect gonadal hormones secretion which results in low TT levels (Nargund, 2015). This indicates that socioeconomic statuses as well as psychological stress influence men's endocrine system and in turn influences the TT level.

5.15 Predictors of Asymmetric Dimethylarginine (ADMA)

In this study, analysis of predictor model revealed that academic qualification and intensity of physical activity were the significant predictors that were more likely to influence ADMA level in ED patients. Men with secondary academic qualification were 4.51 times more likely to have higher ADMA level as compared to men with primary academic qualification. Meanwhile, men who spend less time on physical activities were 2.61 times more likely to have higher ADMA level (p=0.008).

Academic qualification was presented as predictor of demographic factors which influences ADMA level in ED patients. This was probably due to the reason that highly educated men were likely to give greater attention and on their health and have high accessibility for information on their health. Meanwhile, poorly educated men have little knowledge and low understanding of their health. They would also usually neglect treatments for their illness (Momtaz et al., 2014).

Meanwhile, increasing intensity of physical activities could help to decrease ADMA concentration level in ED patients. This finding was consistent with a study conducted by Mittermayer (2005), which reported that constant physical activity significantly decreases ADMA concentration ($p < 0.001$) (Mittermayer et al., 2005). Even though the above mentioned study was conducted on non-ED population, the finding concluded that there is an association between intensity of physical activities and ADMA levels. Physical activities decrease ADMA level by metabolic pathways. It influences the antioxidant status through increased concentrations of oxidants. Thus, improvement of the antioxidant level through increased physical activities influences ADMA synthesis (Mittermayer et al., 2005).

5.16 Strength of Study

This study has several strengths. First of all, this cross-sectional prospective study analysed the combined effect of demographic, clinical and lifestyle factors on increasing severity of ED and biomarkers level in Malaysian ED patients. Unlike most of the ED studies, this study specifically analysed the association of factors with "severity level of ED". Furthermore, the same factors were analysed with two specific biomarkers which are proven to be influencing the severity of ED. The findings of this study could improve knowledge on association of demographic, clinical and lifestyle factors with severity of ED and biomarker level in patients with ED. From this study, the significant predictors influencing severity of ED and biomarker levels were identified. These predictors are important to ensure that ED could be managed and treated more effectively, and a quality pharmaceutical care can be given to ED patients. In addition, this predictor modelling may provide primary guidance to urologists on how ED patients should be treated. Overall, it would potentially improve the management and quality of life of patients with ED. On top of that, the focus of this study on predictors influencing severity of ED and biomarker levels can also serve as a preliminary data for future studies that intend to further investigate on these findings. Since there were scarce studies on the combination of association of demographic, clinical and lifestyles factors with severity of ED and biomarker levels, this new finding could provide useful information for future researchers.

5.17 Limitation of Study

This study possesses several limitations. Firstly, this study was conducted merely at one setting, UMMC due to financial constraints with a small sample size. This could result in the demographic, clinical and lifestyle characteristics of this study population to not possibly represent the actual characteristics of Malaysian population. Hence, large scale prospective studies involving several settings in different states all over

Malaysia are required to validate this finding. Apart from that, the ED clinic is only available on every fourth Friday of a month and it is uncertain to meet all patients from this clinic because patients might miss their appointments and this could lead to loss of samples. Thus, most of the samples are obtained from medical, surgery or eye clinics. Biased selection might occur because convenience sampling was applied for control group. Few patients refused to participate since this study is based on a sensitive issue and they were found to be uncomfortable during the interview session. Besides that, language became a major barrier for this study. Both the questionnaire and interview session was done in either English or Malay language based on patients' preferences. The main reason to decline from participating in this study was due to the difficulties to understand either one of the languages that were used by the researcher.

CHAPTER 6: CONCLUSION

In conclusion, the purpose of this study was to assess and relate the combination of demographic, clinical and lifestyle factors which were associated with the severity of ED and biomarker levels. The binary regression result concluded occupation and TT as the significant predictors causing severe ED among Malaysian ED patients. Demographic and clinical factors dominate lifestyle factors in determining predictors of severity of ED in Malaysian patients. Therefore, lifestyle factors did not really influence severity of ED in Malaysian patients. It shows that Malaysian patients generally live a healthy lifestyle practicing good ethics in term of physical activeness, smoking and alcohol consumption.

Meanwhile, demographic and lifestyle factors predominate clinical factors in influencing TT and ADMA levels. Occupation or employment status was the only variable that served as a significant predictor which influenced TT level in ED patients. Pensioners and those unemployed were most likely to have low TT level compared to other groups who were employed and economically stable. Those unemployed were more likely to have unhealthy lifestyle which has been linked to increase in morbidity levels. Besides, psychological and lifestyle effects of unemployment could significantly impact longer-term health accompanied with increase in stress level and eventually lead to imbalance of hormone levels.

On the other hand, academic qualification and intensity of physical activity served as predictors which influenced ADMA level in Malaysian ED patients. Educated men commonly have greater knowledge and value their health highly. They constantly look for the guidance of doctors on their health and communicate effectively with them. However, poorly educated individuals were unaware of their health status due to less knowledge on diseases and its symptoms and the poor quality of communication with

physician contributes to the high percentage of health issues among this group. Besides, regular exercise helps promote and maintain proper hormonal balance in men by maintaining a healthy weight in order to decrease ADMA level by reducing insulin resistance. Additionally, exercise may help to relieve stress. Stress plays a pivotal role in ADMA pathophysiology by managing PRMT/DDAH expression and NO synthesis and leading to ED. Thus increase in intensity of physical activity subsequently influence ADMA level and decrease severity of ED.

Physician should consider ED patients' demographic background in treating them and proper counseling should be given for those with minimal knowledge with unstable employment and financial status. As per the present results, there is a reasonable need to investigate the TT level, academic and occupational background and lifestyle of patients when they present with severe ED. Urologists or ED experts should examine patients' TT levels and add the necessary improvements in treating ED patients. Besides, urologists also need to educate ED patients on the importance of a healthy lifestyle and give priorities for their health issues regardless of their occupation and academic background and help them to live a quality life. Identification of these predictors influencing severity of ED, TT and ADMA level may help to increase our knowledge on risk factors of ED. Thus, these predictors may serve as new indicators in providing primarily guidance to urologists or physician and ensuring ED can be managed and treated more effectively.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

1. Title : Oral drug treatments in patients with erectile dysfunction and multiple comorbidities: a retrospective observational study

Journal : BMJ Open

Authors : Zaman Huri Z, Lian Choo T, Sulaiman CZ, Mark R, Abdul Razak AH

2. Title : Association of Psychological Factors, Patients' Knowledge and Management amongst Patients with Erectile Dysfunction

Journal : Patient Preference and Adherence

Authors : Zaman Huri Z, Mark R, Abdul Razak AH

3. Title : Demographic, Clinical and Lifestyle Predictors for severity of Erectile Dysfunction and Biomarkers level in Malaysian Patients (Accepted for publication on 30th January 2018

Journal : Brazilian Journal of Pharmaceutical Science

Authors : Mark R, Zaman Huri Z, Abdul Razak AH

4. Poster presentation at 23rd Malaysian Urological Conference MUC 2014, 21- 23 November 2015 at the G Hotel, Penang, Malaysia

Title : Association of potential biomarkers with severity of ED and oral drug treatment in ED patients with multiple comorbidities.

Organizer : Malaysian Urological Association