Microvascular Pathogenesis of Erectile Dysfunction via RhoA/Rho-kinase Pathway in Diabetes Mellitus.

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ABSTRACT
Diabetes Mellitus DM) is a chronic, non-communicable metabolic disorder characterized by a high glycemic index which is caused either by impaired secretion of insulin or its function or both. With 5.2 million fatalities globally attributable to diabetes and an overall mortality rate of 82.4 per 100,000 people. Chronic hyperglycemic conditions lead to microvascular complications affecting the kidneys, eyes, and neurological system. Sexual dysfunction also occurs as a result of chronic hyperglycemia both in men and women. Premature ejaculation and erectile dysfunction are some of the major sexual disturbances associated with diabetes. %). The prevalence of erectile dysfunction varied among different age groups. Between the ages of 40 and 49, 2% to 9%, 60 to 69 years old, it then rises to 20 to 40%, more than 70 years old it then rises between 50% and 100%. Female sexual dysfunction (FSD), which includes dyspareunia, blocked intercourse, vaginal laxity, and diminished sexual desire, arousal, or orgasm, refers to a deviation from normal sensation and/or function during sexual activity. Many mechanisms are attributed to Erectile dysfunction like the eNOS pathway, oxidative stress, NADPH oxidase, etc. In this review article we particularly focus on RhoA/Rho-kinase pathway and how it disturbs the normal Erectile function in Diabetes mellitus and also the current Treatment options available for Erectile dysfunction.

KEYWORDS: Diabetes Mellitus, Male Sexual Dysfunction, RhoA/Rho-kinase pathway.

INTRODUCTION:

Diabetes Mellitus DM) is a chronic, non-communicable metabolic disorder characterized by high glycemic index which is caused either by impaired secretion of insulin or its function or both.(1) With 5.2 million fatalities globally attributable to diabetes and an overall mortality rate of 82.4 per 100,000 people, it is currently the seventh greatest cause of death in both the USA and the world. (2)

Genetic predisposition and persistent intracellular hyperglycemia eventually impair the microvasculature, resulting in issues mostly involving the kidneys, eyes, and neurological system. End-stage renal disease (ESRD) is mostly brought on by diabetic nephropathy and Diabetic retinopathy (DR) is the primary cause of blindness in the industrialized world.(3)

Sexual dysfunction also occurs as a result of chronic hyperglycemia both in men and women.(4)(5) Up to 25% of T2DM patients who have just received a diagnosis have reportedly already had one or more problems. Premature ejaculation and erectile dysfunction are some of the major sexual disturbances associated with diabetes.(6) The pathophysiology of erectile dysfunction (ED) is exceedingly complex and is thought to be caused by a combination of vasculopathic, neuropathic, and hormonal abnormalities in diabetic individuals.(6)

Erectile dysfunction is the inability to obtain and/or maintain an erection strong enough to allow for satisfying sexual activity.(7) It is a prevalent clinical condition that primarily affects males over the age of 40.(8) The development of erectile dysfunction has been connected to a number of common lifestyle factors, including obesity, little or no physical activity, and symptoms of the lower urinary tract, in addition to the traditional causes of the condition, such as diabetes mellitus and hypertension.(4)

EPIDEMIOLOGY OF SEXUAL DISTURBANCES IN DIABETES

Diabetes mellitus has emerged as one of the most urgent and widespread problems in recent years. It is now the seventh leading cause of death in the USA as well as the rest of the world, accounting for 5.2 million deaths globally and having a mortality rate of 82.4 per 100,000 people.(2)

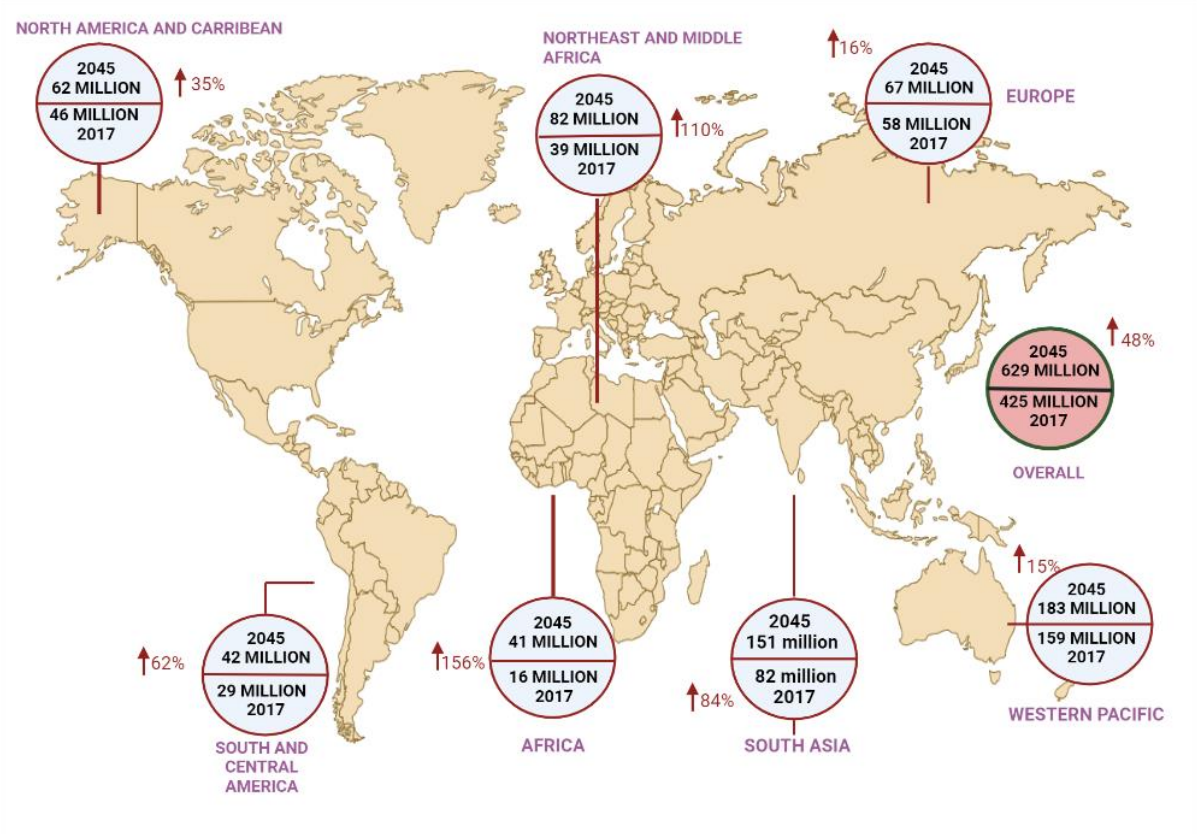


Fig. 1 Number of people with diabetes worldwide and per region in 2017 and 2045.

Over 62 million diabetic people are thought to reside in India as of 2014. Be a result, India is referred to as the "world's diabetes capital". According to a research by the Indian Council of Medical Research, the majority of the country's affected people are from Maharastra (9.2 million people) and Tamil Nadu (4.8 million). This number may be distorted because many rural areas lack access to diabetic screening technologies, which makes underreporting possible.(9)

In men under the age of 40, erectile dysfunction was common (1–10%). Prevalence of erectile dysfunction varied from between the ages of 40 and 49, 2% to 9% in men. In men 60 to 69 years old, it then rises to 20 to 40%. Between 50% and 100% of males over the age of 70 have erectile dysfunction.(8) People with diabetes experience ED 10–15 years earlier than patients without diabetes. (7)

Ethnicity was not a factor in ED prevalence. The majority of the traditional risk factors for ED, such as diabetes, hypertension, and ageing, play a similar role in both the United States and the rest of the world. (10)Premature ejaculation and genital size are newly identified ED risk factors. Alcohol consumption and smoking frequency were not associated with a greater prevalence of ED, but smoking length was.(11) An estimated 50–75 percent of diabetic males develop ED to some extent.(11)

SEXUAL DISTURBANCES IN MEN AND WOMEN.

Diabetes-related metabolic issues can result in a number of consequences, including problems with the male and female reproductive system.(12)

Along with the microvascular problems of diabetes mellitus (DM), such as nephropathy, retinopathy, and neuropathy, macrovascular complications—such as coronary artery disease, peripheral vascular disease, and carotid artery disease—also become more common as the duration of diabetes increases.(2)

Female sexual dysfunction (FSD), which includes dyspareunia, blocked intercourse, vaginal laxity, and diminished sexual desire, arousal, or orgasm, refers to a deviation from normal sensation and/or function during sexual activity(13)(14).

According to a global study of 13 882 women between the ages of 40 and 80, 39% of sexually active women reported having at least one issue with their sexual activity.(15)

The prevalence of FSD is about 30% in women. This prevalence increases from 54% to 64% in 595 diabetic women aged between 35 and 70.(16)

SD appears to be more strongly correlated with psychological factors and low mood in the diabetic female population than with metabolic changes. Sexual function may be negatively impacted by hyperglycemia, neurovascular changes, psychological disorders, or persistent genital infections, which are common in female diabetic patients.(17)

Male sexual dysfunction majorly includes erectile dysfunction, peyronie’s disease, premature ejaculation etc.,

Peyronie’s Disease

Peyronie's disease (PD) is characterized as improper penile injury healing. Plaques develop in the tunica albuginea as a result of excessive collagen deposition. The tunica albuginea becomes stiffer due to collagen plaques, which can cause ED, penile deformity, abnormal penile curvature, penile pain,(18) and painful erections.(19)(20) It is a widespread disorder with an estimated prevalence of variation within certain groups from 0.5% to 20.3%.(21)

The most frequent inciting event is thought to be sexual activity, during which patients are thought to experience PD. PD is diagnosed incidentally during medical visits for other primary concerns, such as prostate cancer screening (reported 8.9% prevalence) or erectile dysfunction (reported 16% prevalence) may suffer from penile buckling while erect or semi-erect, which would harm the penile shaft's microvascular network.(22)

Premature Ejaculation

Premature Ejaculation (PE) is defined as the inability to postpone ejaculation long enough to experience intimacy, which shows up as either ejaculation occurring before or right after the commencement of intercourse or ejaculation occurring when there is not enough erection to allow for intercourse**.**(23) (24)PE is actually of two types: lifelong PE and Acquired PE.

Lifelong PE is also termed as primary PE and is characterized with ejaculation within seconds or a minute since their first sexual intercourse and this persists for rest of their lives.(22) (25)

Men with procured PE begin to complain about their condition after never previously having done so. They typically ejaculate immediately or within three minutes. Their complaints of

PE are frequently caused by somatic issues(26,27). As a result, it is also known as secondary PE.(22)

The epidemiology of PE differs greatly between regions of the world. According to different researches, the prevalence of PE among sexually active men fluctuated from 20% to 75%.(23)(28)

The inability to achieve or maintain a hard adequate erection for satisfying sexual interaction is called as erectile dysfunction.(29) (30)

In order to achieve a penile erection, the penile vasculature needs to be dilated, the corporal smooth muscle needs to be relaxed, the intracavernosal blood flow needs to be enhanced, and the veno-occlusive function needs to be normal. Eighty percent of ED cases are brought on by penile vascular disorder, which is brought on by alterations in the nitric oxide/cyclic guanosine 3'5'-monophosphate (NO-cGMP) pathway connected to endothelial dysfunction.(29)

MICROVASCULAR PATHOGENESIS OF ERECTILE DYSFUNCTION

RhoA/Rho-kinase pathway

The small GTPase RhoA can alternate between an active, GTP-bound state and an inactive, GDP-bound one.(31) GTPases are a large family of hydrolase enzymes that bind to the nucleotide guanosine triphosphate (GTP) and hydrolyse it to guanosine diphosphate (GDP)

Numerous cell-surface receptors, such as the cytokine, tyrosine kinase, adhesion, and G-Protein Coupled Receptors, are frequently involved in the activation of Rho family proteins.(32) (33)

Guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) are two proteins that control the activity of RhoA. (31)

Regulatory proteins for Rho-like GTPases like GEFs are a large family of rapidly growing proteins that has Dbl-Homology domain (DH) with common motif. Dbl's 29% sequence homology with the Saccharomyces cerevisiae cell division cycle protein Cdc24, which by genetic study was positioned upstream of the yeast small GTP-binding protein Cdc42 in the bud assembly pathway, provided the first hint as to its role as a GEF.(34) GEFs can activate RhoA by promoting the exchange of GDP for GTP.(24) RhoA GTPases can be activated by many Guanine nucleotide Exchange Factors in in-vivo and in-vitro studies.(35)

GAPs can inactivate RhoA by accelerating the hydrolysis of GTP to GDP. Rho protein intrinsic GTPase activity is boosted by GTPase-activating proteins (GAPs), which are rather extremely slow.(32)

By biochemically analysing cell extracts with recombinant Rho, a prototype GAP protein specific for the Rho family GTPases was isolated. In vitro tests on this protein, known as p50Rho-GAP, revealed GAP activity toward Rho, Cdc42, and Rac.(34)

Guanine dissociation inhibitors (GDIs) likewise stop the activation of RhoA via a number of different methods.(31)

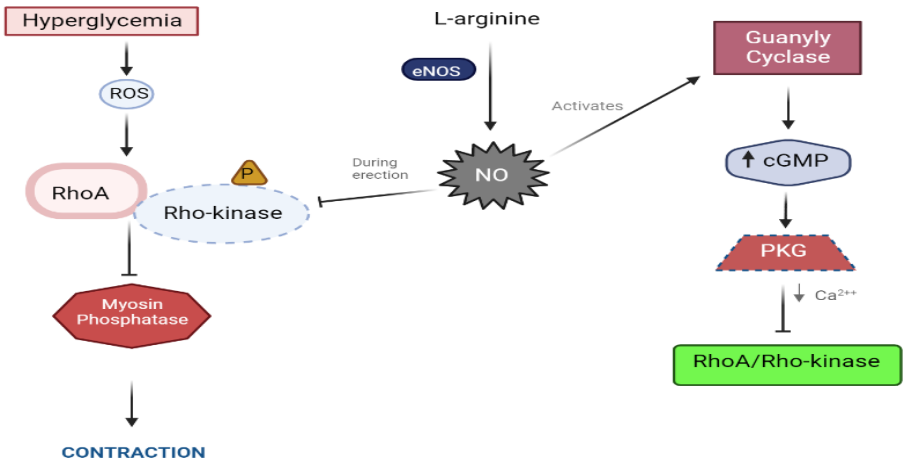
The ubiquitously produced protein Rho-GDI was isolated as a cytosolic protein and preferred to connect with the GDP-bound form of RhoA and RhoB and so block the dissociation of GDP, was the first GDI discovered for the members of the Rho family.(34) The translocation

of the Rho GTPases across membranes and the cytoplasm appears to be largely dependent on the Rho GDIs.(34)

Many receptor systems such as α-adrenoreceptors, angiotensin - II, Sphinghosine-1-phosphate receptors activates GEFs which further activates RhoA.(31) Several downstream targets are available for RhoA and yet ROCK (Rho-associated coiled-coil protein kinase) is most prominent among all of them.(31) p160/ROCK or Rho kinase interactions with Rho resulted in a small increase in the kinase activity.(23)

The production of stress fibres and focal adhesions is aided by the expression of full-length ROKa and its amino-terminal half. This response needed kinase activity but not the Rho-binding domain.(34)

The pathophysiology of diabetic ED is linked to both decreased vasodilation and increased erectile tissue contractility. The RhoA/Rho-kinase pathway is a vasoconstrictor mechanism and is active in the penis.(36)



RhoA/Rho-kinase Activity in Corpora cavernosa smooth muscle cells and in non-penile vascular beds.

Myosin light chain phosphatase subunit 1 is a regulatory enzyme of myosin phosphatase that targets and inhibits its activity by RhoA-activated Rho-kinase via phosphorylation at Thr-696, and increasing smooth muscle contraction.(36) NO blocks this route during erection. Increased levels of cGMP are the result of soluble guanylyl cyclase being activated in corporal smooth muscle cells by NO's binding to its heme moiety. The corpora cavernosa's vascular smooth muscle cells relax under the influence of NO when cGMP-dependent protein kinase (protein kinase G) is activated.(24) This is because cGMP-dependent protein kinase reduces intracellular calcium and inhibits the RhoA/Rho-kinase pathway.(36) (31)

The RhoA/Rho-kinase pathway inhibits Akt-dependent eNOS activity/phosphorylation at Ser-1177,105 in human umbilical vein endothelial cells, revealing a further mechanism of interaction between eNOS-mediated relaxant and RhoA/Rho-kinase-mediated contractile pathways in the penis.(36) (37)There is indeed communication between the Rho/ROCK and PKB pathways, and this communication negatively controls PKB activation.((38)

Rho/ ROCK's inactivation of PKB is what prevents eNOS phosphorylation from happening but not eNOS gene expression.(38)

Recent research indicates that oxidative stress is a key mediator of diabetic problems. The formation of ROS in mitochondria, which impairs mitochondrial function, is triggered by an excessive load of glucose in cells that are sensitive to hyperglycemia, such as endothelial cells.(3)(39)In nonpenile vascular beds, ROS promote enhanced migration of RhoA to the plasma membrane and its activation, which in turn activates the RhoA/Rho-kinase pathway. (36)

NAD(P)H oxidase is stimulated by rho-kinase, which results in an increase in ROS generation.(40)(41)

TREATMENTS FOR ERECTILE DYSFUNCTION

ED significantly lowers a man's quality of life by causing anxiety, melancholy, loss of self worth, all of which leads to sexual dysfunction.(42)Age, coronary artery disease, obesity, smoking, depression, hypertension, past pelvic surgery, spinal cord injury, and other psychological issues are also risk factors for ED.(43) A referral to a mental health expert is advised by American Urological Association (AUA) recommendations as an additional therapy for the treatment of ED5.(43) A thorough history and clinical examination pertinent to psychological and organic causes are necessary for the clinical assessment of ED.(42)

The IIEF is a validated subjective score that has excellent sensitivity and specificity for ED, and it is utilised as a benchmark worldwide in the clinical investigation of traditional and surgical therapies for ED.(42)

SCORE	ED CLASSIFICATION
≤ 7	Severe
8-11	Moderate
12-16	Mild to Moderate
17-21	Mild
≥ 22	No ED

Table no - 1, ED Score and it's Severity.

Oral medications

Phosphodiesterase-5 inhibitors are the most common pharmaceutical treatments for ED because they gives out nitric oxide (NO). (44)Guanylate cyclase (GC), which turns guanosine triphosphate (GTP) into cyclic guanosine monophosphate, is stimulated by NO (cGMP). After that, cGMP causes smooth muscle relaxation, which permits blood to flow into the penis, which causes an erection. Typical examples include sildenafil citrate (Viagra)(45) and tadalafil (Cialis).(42)(43)

Intracavernosal injection

For the treatment of ED, intracavernosal injections (ICIs) are an alternative to oral medicines. The medication is directly injected into the penile corpora at the lateral base of the penis(46) during this procedure.(47)

Prostaglandin E1 (PGE1), the drug that is most frequently administered, promotes cyclic adenosine monophosphate (cAMP) to cause smooth muscle relaxation and encourage erections.(43)

Intraurethral suppositories

Additionally, an intraurethral suppository of alprostadil is given. Some people may choose this method of delivery if they want to stay away from oral or injectable drugs. While erectile function is improved by intraurethral alprostadil compared to placebo, it is not as effective as ICI. Side effects of using this drug is penile and urethral pain.(43)(48)

VED

The vacuum-assisted erectile device (VED) sucks blood into the penis to generate engorgement and erection.(49) It is placed over the penis and pumped to create a vacuum. The penis is then wrapped in a band to keep the erection in place, and the band is subsequently unwrapped to let the penis go back to its flaccid state.

Long-term use declines because many patients find the devices uncomfortable to use(50) and worry that wearing a restrictive ring will cause pain or temporarily alter their penile sensation.(43)

Novel Therapies

Platelet-rich plasma

Growth factors produced by platelets are crucial for the development of new blood vessels as well as the healing of wounds. PRP injections intravenously have been proven to improve erectile function in cases of neurogenic ED in animal models. This has been applied to human studies, and a recent study of four ED patients treated with PRP showed improved erectile function without any severe adverse events.(43)

Stem cell Therapy

The use of stem cells to treat ED has recently attracted interest. Injection of stem cells after prostate radiation in animal studies has demonstrated the restoration of erectile function through the regeneration of cavernosal nerves. Intracavernosal stem cell transplants for the treatment of ED have showed promising results in minor phase 1 studies in people in terms of their acceptability, safety, and effectiveness.(43) Many other Novel therapy are under investigation and research has to be done in future to come up with more drugs for ED.

CONCLUSION

The prevalence of T2DM microvascular problems is significantly increasing. The pathophysiology of the majority of these issues, however, is still not well known. For the early detection of these problems, more sensitive biomarkers are required. Interventions that could revert the pathophysiologic processes involved in the onset and progression of these disorders are also required.

It is known that one of the main factors causing ED in people with diabetes is endothelial dysfunction. Other mechanisms are also involved in pathogenesis of Erectile dysfunction like supressed eNOS activity/ epression, eNOS uncoupling, increased Oxidative stress, NADPH oxidase and many more. Diabetes-related ED may also be accompanied by endocrine abnormalities such low testosterone and alterations in hormone receptors. A unifying aspect for all these mechanisms is Endothelial dysfunction. Ineffective vasorelaxation, increased

vasoconstriction, an increase in the production of free radicals, and other endothelial injury-promoting factors lead to endothelial dysfunction.

The first effective class of oral therapies for erectile dysfunction—PDE5-Is—was created as a result of significant advancements in our understanding of the pathophysiology of erectile dysfunction and the physiology of erection. Further investigations should be done to come up with many therapeutic agents for Erectile dysfunction.

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