ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 11 | November - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ENDOPLASMIC RETICULUM STRESS AND METFORMIN: BOON OR BANE IN FEMALE COVID-19 PATIENTS

Gynecology

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ABSTRACT

The year 2020 witnessed the upsurge of SARS-Cov2 the name given to virus causing COVID-19 pandemic. Scientists thought that the virus was limited to animals but however first human form was found in 1960 which was predominantly limited to children causing lung infections. After the pandemic several studies revealed it to be male dominated since more than 74% males got infected as compared to females. This is also correlated due to high prevalence of co-morbidities such as cardiovascular diseases, hypertension etc. in males. Due to purbutations caused by Cov-2 spike proteins in endoplasmic reticulum, there is down regulation of signaling pathways creating endoplasmic reticulum stress. This in turn causes more complications if a SARS-Cov2 patient has co morbidities. Estrogen a major sex hormone in females plays a protective mechanism by upregulation of JNK 1/2-NFκβ pathway or Calcineurin/NF-AT3. On one hand inflammation or high levels of reactive species in ovaries, elevates oxidative stress which in advanced stage causes ER stress in granulosa cells. This can be one of the causes of egg arrest in estral stage in ovaries leading to PCOS/infertility. Metformin on the other hand acts therapeutically in treating women with infertility or PCOS. It acts as an adjuvant and manages insulin dependency of the body by activating phosphorylation of AMPK. It also lowers ER stress by down regulating chaperons Glucose regulated protein 78(GRP78) and PDI (Protein disulphide isomerase). Metformin if administered in PCOS induces ovarian theca cells by suppressing androstenedione along with decreased FSH stimulated enzyme activity of 3β-HSD, StAR, aromatase and CYP-11A1. Metformin and Estrogen works hand in hand, when microbiota of gut is healthy, and should not be ignored while treating woman SARS-Cov2 patient. Treatments to women SARS-Cov2 patient must be given by understanding a broader picture of their biological makeup.

KEYWORDS

SARS-Cov2, ER Stress, PCOS, Metformin, Female mortality

BACKGROUND:

According to one of the leading corona virologists, term corona was coined by International committee on taxonomy of viruses while focusing on botanical word corona from sunflower, according to arrangements of its petals which are radiated or crown shaped and might resemble surface receptors of corona viruses(1). The first time Human corona viruses came into limelight when they were first characterized in the 1960s (2). These viruses are not only limited to humans, they can infect various animals like birds, cows, pigs, mice; causing upper respiratory tract infections, diarrhea, hepatitis or neurological disorders (3). The early Human Corona virus was considered as pathogen responsible for a considerable amount of infections in upper respiratory tract among children. 7 years later it was found that around 41% of adults and only 0.6 % of children were infected with its strain 229 E, when their compliment fixation antibody tests were performed(4). In year 2003, 5 new human corona viruses were identified, including the severe acute respiratory syndrome corona virus, commonly abbreviated as SARS-1 (Severe Acute Respiratory Syndrome) which caused significant morbidity and mortality throughout world (2). They are typically RNA virus, enveloped and in humans its multiple variants existed till date causing diseases like SARS-CoV, Middle East Respiratory Syndrome (MERS-CoV) and now global pandemic causing Novel SARS- COVID-19(5). The newly emerged Corona virus SARS-CoV-2 created a respiratory illness which has spread throughout the world within months causing a global pandemic i.e. Corona virus disease or abbreviated as COVID-19 as the matter of fact it was firstly observed in year 2019 in Wuhan, China (6). This disease got spread in around 213 countries and territories affecting more than 235 million people with total death toll of 4.8 million and counting (7).

Women and COVID-19:

Sexual Predisposition:

A gender based study by Shabbir S et al (8) suggested SARS-Cov 2 to be gender biased, as 74% of total infected cases were male and only 26% females got infected with this virus. Another study by Remuzzi A et al (9) stated that male to female ratio of total population infected by SARS- CoV-2 had a drastic difference of 80/20. In China men were more affected by SARS-CoV-2 as compared to women was also proved by Cai H(10) by revealing contradictory data of percentage of men as compared to women stating in some regions male to female ratio was equal, in some regions around 58% were men and whereas 67% critically ill patients were males. This can be either due to higher prevalence of diseases in men such as diabetes (11), peripheral arterial disease (12), hypertension (13) or cancer (14) etc. or is it because of complex hormonal pathway platform making it little harder for virus to survive in female body.

Role of Endoplasmic stress in COVID-19:

In year 2002 previously established virus SARS-CoV-1 emerged and showed distinct features like 9 unique open reading frames (orfs) (15), out of which third frame is largest and produce a protein called as 3a protein, made up of 274 amino acids. This protein is exclusive part of virus and expressed in both infected and transfected cells (16).The typical S protein in all coronavirus is a homotrimeric spike glycoprotein (S1 and S2 subunit in each viral spike monomer) giving its envelope a decorative look. These glycoproteins helps in binding to their cellular receptors, later triggering the cascade of events which eventually fuse viral and cellular membranes and making it possible for viral proteins to enter correctly (17). Viral genome will conquer host genome and order it to reproduce its viral proteins in abundance to be utilized in viral propagation.

As endoplasmic reticulum is the site of protein translation and its processing, it is most likely to be exploited by this RNA virus for its insertion. Pertubations in protein synthesis and folding gives a signal to up-regulate cellular metabolism and protein formations which is directly regulated and monitored by Endoplasmic reticulum (18). Cells own machinery will be loaded with unfolded or nascent proteins which will abruptly down regulate signaling pathways and ultimately cause endoplasmic reticulum stress (19).

Unfolded protein response (UPR) is initiated with this down regulation and perturbations of ER processing which later turns on apoptosis to balance and normalize its normal functioning. Triple A response of body - adaptation, alarm and apoptosis can underline the mechanism or

ER stress and turning on UPR (20). ER stress is also associated with number of diseases such as diabetes, neurodegenerative diseases like Alzheimer's disease, or ischemia, thus intimating a direct link of downfall of health when infected with SARS-CoV-2 in previously compromised patients, further propelling them into critically ill category (21).

Estrogen and Endoplasmic Reticulum stress:

ER stress starts a cascade in the cells, initially it starts UPR mechanism which induces the release of *cytochrome c* from mitochondria, and later triggers the apoptosis of βcells(22). It was also presented that both mitochondria and endoplasmic reticulum in a cell are important because they regulate metabolism by controlling ATP production, cellular redox by controlling ROS, pH, Ca2+ signaling, and final cell death (23). ER-α deficient muscle cells also showed impaired autophagy signaling. Autophagy is parallel induced by cellular stress and elevated lipids and ERα is also critical in the regulation of β-cell mitochondrial dynamics and mitophagy signaling (22).

Estrogen is the major steroid sex hormone secreted in both males and females contributing to their reproductive health and controlling secondary sexual characters (24). Cardiac cells also express Estrogen receptors (ERs). Some vivo and in vitro studies suggested that 17 beta-estradiol (E2) contribute to cardioprotection by preventing apoptosis of cardiomyocytes, alleviating left ventricular hypertrophy and cardiac fibrosis in women in postmenopause(25). Cardiotoxicity is inhibited by Estrogen through upregulation of Estrogen receptors via various mechanisms like JNK 1/2-NFκβ pathway or Calcineurin/NF-AT3(26).

In breast cancer proliferation ER stress is high due to uncontrolled cell growth and proliferation. Levels of hormones, metabolites, cytokines/chemokines are altered due to this stress and further enhance the growth BrCA cells. In ER-α, a rare inactivating mutation i.e. *ESR1*, along with this polymorphism at this Loci may be associated with higher risks of diseases like obesity, artherosclerosis or type-2 diabetes, independent of hormonal status of the women (27). Thus understanding ER stress can be a therapeutic tool to underline status of women health and its ability to respond to pathogenic invasions too.

Granulosa cells and Endoplasmic Reticulum stress:

Poly cystic ovarian syndrome (PCOS); a lifestyle disorder is either an endocrine or metabolic or combination of both, where due to androgen excess or insulin resistance, there is an egg arrest at follicular stage (28). To understand pathogenesis of PCOS, it must be clear that oxidative stress due to reactive species or inflammation in the ovaries as substantially the same like reactions causing UPR regulation leading to ER stress in granulosa cells. It can be either said as ER stress causes activation of several cascades of UPR causing accumulation of unfolded proteins and tissue fibrosis which makes eggs seizes at various stages, procuring PCOS and its associated symptoms (29).

Patients suffering with infertility had elevated levels of palmitic acid (PA), a 16 C saturated fat obtained from plants, animals or microorganisms and sustained toxic effect on granulosa cells. In a study when methyl thiazolyl tetrazolium (MTT) assay, flow cytometry followed by western blotting was performed on granulosa cells of mouse. With varying range of PA , there was reduction of cell viability along with induction of apoptotis, there was enhanced expression of apoptosis related genes such as Caspase 3, B-cell lymphoma (bcl-2) associated X protein (BAX), higher expression of ER stress marker genes such as glucose regulated X protein 78(GRP78) and CCAAT/enhancer binding protein homologous protein (CHOP) (30).

Metformin and Endoplasmic Reticulum stress:

Cell injury induces mitochondrial dysfunction along with ER stress as mitochondria and ER are closely related cell organelles. Disruption of mitochondrial electron transport chain becomes a stepping stone for oxidative stress. It becomes evitable to propagate the evidence that ER too induces ROS generation or vice versa. Metformin, an adjuvant which works wonders for reducing diabetic effects in the body also protects diabetic patients from mortality resulting of CVD. AMP-activated protein kinase is an energy sensor molecule, regulates mitochondrial metabolism and redox reactions. Metformin activates AMPK by phosphorylation leading to selective permeability in cardiac muscles (31).

Two ER chaperons namely Bip also known as Glucose regulated protein 78(GRP78) and PDI (Protein disulphide isomerase), are parts of UPR gene elements and inducers of ER stress by lipotoxicity. Metformin reduced the amount of these chaperons to optimum levels effecting ER stress. It was also found out that metformin also reduced the CHOP expression and apotosis inducer (32).

PCOS and metformin:

Poly cystic ovaries usually appear when there is hyperandrogenemia or insulin sensitization. Metformin reverses metabolic effects of PCOS and also harmonize hormonal balance to induce fertility (33). Some of the studies collected evidences over reduction of PCOS effects when metformin is advised with Clomiphene citrate as they worked additively (34). Metformin was found capable to reducing BMI and insulin levels (fasting) in patients of PCOS which enhances their fertility (35).

Advantages of Metformin on women health are numerous and exclusively studied. The most effective one is reducing or neutralizing the excess insulin which predominately affects ovarian health. This is conclusive fact that insulin induced various steroidogenic enzymes inside ovary, such as 3β-HSD, StAR,CYP-17. As insulin sensitivity goes up, metformin lowers down the CYP-17 activity. Ovarian theca cells forming androstenedione are suppressed by metformin along with lowering down of FSH stimulated enzyme activity of 3β-HSD, StAR, aromatase and CYP-11A1 in PCOS patients (36)

Estrogen and Metformin:

Flora and fauna of the gut's micro-environment plays an important role in regulating hormones like estrogen and essential levels of estrogen itself grooms healthy micro environment. Deconjugation of estrogen is possible due to gut biome's secreting enzyme glucuronidase . Estrogen eventually binds to its receptors and downstream regulation of hormone occurs. Dysbiosis is corrected by fecal microbiome transplant, bariatric surgery or chemically by metformin due to its inducing effects (37). Metformin as a first line of treatment in PCOS induces expression of Estrogen receptor –α, while metformin when combined with low doses of flutamide decreases intestinal androgen receptors with upregulated ER-α mRNA expression (38). In the era of Covid-19 metformin is thought have immune-modulatory effects in reducing the inflammation in women with obesity or Type 2 diabetes mellitus. This effect is attained by reducing TNF-α due to sex specific hormone response (39). Reduced Covid-19 mortality was associated in women when administered with metformin by Cox proportional hazards and propensity matching (40). Figure1.

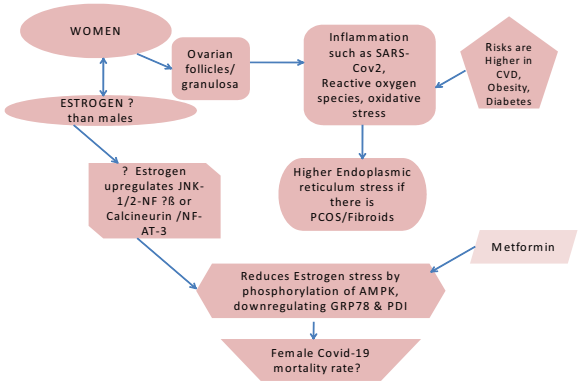

Figure1. The role of estrogen in controlling endoplasmic reticulum stress and how it work collectively in lowering mortality rate in Covid-19.

CONCLUSION:

Connecting loopholes for successful SARS-Cov-2 treatment in women

When a woman suffering with SARS-Cov2 arrives, her complete history must noted down and carefully evaluated and ruled out for PCOS, Post/premenopausal, Diabetes or CVD. Even parameters like BMI or lipid profile must not be neglected. With advancement of disease, ER stress might pose oxidative stress or fibrosis of ovarian/uterine tissues. SARS-Cov2 induces cardiac myopathy due to up regulation of cardiac tissues/ turning on of its UPR gene elements. Corona virus invade glucose metabolism by inhibition of ATP production or Ca+ signaling, along with sensitizing insulin.

Metformin on the other hand restore body's response to insulin for normal functioning. Thus if a women SARS-Cov2 infected patient with history of PCOS, diabetes or Cardio Vascular Disease could be administered with metformin as an adjuvant for lowering down ER stress so that there is up regulation of anti-apoptotic system causing reversal of damage by releasing factors like TNF-1, IL-6, Bcl-2 etc. This will help the patient to recover more efficiently as compared to approved line of treatments. It is thus suggested that further investigations may be done to explore whether metformin/ flutamide can be given as side line treatments while attending a SARS-Cov-2 infected female to reduce ER stress.

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