

Effects of obesity-related inflammatory markers on psychosomatic manifestations of premenstrual tension syndrome: towards better therapeutic outcomes (An original article)

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Abstract

Background: Obesity-induced inflammation facilitates depression and premenstrual tension syndrome. Hypoxia is a common feature of inflammation. Hypoxia inducible factors adapt cells to low oxygen tension and inflammation.

Objectives: We aimed to see how obesity, along with Amiloride, Hydrochlorothiazide, Metformin, calorie restriction, and walking exercise, affected psychosomatic characteristics of premenstrual tension syndrome, during a six-month period.

Patients and methods: A prior ethical committee approval and informed patients' consent were taken. This study was performed in Tanta University, Egypt from May 2019 to December 2019. This study aims at evaluating the effects of obesity-induced inflammatory mediators on psychosomatic effects in women having premenstrual tension syndrome. Effects of combined therapy using (Metformin, Amiloride. Hydrochloride/ Hydrochlorothiazide, caloric restriction, half an hour of walking exercise per day, and Vitazinc capsules) treatment was compared to the same combined therapy including Royal vitamin G treatment (instead of Vitazinc) on alleviating psychosomatic manifestations of premenstrual tension syndrome. Sixty obese women having premenstrual tension syndrome were categorized into younger age group (18-39 years) and older age (40-48 years) versus a non-obese age-matched control

group. Body mass index in addition to serum tumour necrosis factor- α (TNF- α), hypoxia-inducible factor-1 α (HIF-1 α) and receptor activator of nuclear factor- κ B ligand (RANKL) were assessed. Related psychosomatic manifestations of premenstrual tension syndrome (edema, anxiety, and fatigue) were also assessed.

Results: Obesity significantly increased serum TNF- α ($p < 0.01$), HIF-1 α ($p < 0.01$) and RANKL ($p < 0.01$). Obesity-induced biochemical effects were higher in older obese women than younger ones. Obesity significantly exaggerated the severity of investigated psychosomatic manifestations ($p < 0.001$). Both combined therapies (including either Vitazinc or Royal vitamin G) significantly and dramatically decreased the percentage of cases having psychosomatic manifestations ($p < 0.001$) that was closely related to the decreased serum biochemical parameters.

Conclusion: Combined therapy containing Royal vitamin G significantly improved serum biochemical parameters and psychosomatic manifestations better than combined therapy containing Vitazinc.

Keywords: premenstrual tension syndrome, psychosomatic manifestations, anxiety, royal vitamin G and Vitazinc.

Introduction

Premenstrual tension syndrome (premenstrual syndrome) includes behavioural, psychological, and physical symptoms recurring cyclically during the luteal phase of the menstrual cycle (Delaram et al., 2011). Chronic inflammation was reported to be implicated in the etiology of depression and other disorders that share common features with premenstrual tension syndrome (Bertone-Johnson et al., 2010; Bertone-Johnson et al., 2014; Bertone-Johnson et al., 2016). Hypoxia is a common feature of inflammation. Hypoxia inducible factors adapt cells to low oxygen tension and inflammation (Kerber et al., 2020). A study by Foster et his co-workers reported that women having premenstrual tension syndrome exhibited higher

concentrations of the inflammatory markers interleukin (IL)-1 β , IL-6, and IL-8 and tumour necrosis factor- α (TNF- α) than those without premenstrual syndrome (Foster et al., 2019).

Estrogens inhibit the production of receptor activators of nuclear factor κ B ligand (RANKL). RANKL is vital for osteoclast differentiation and recruitment (i.e. helps bone resorption) as well as increased interleukin IL-1, IL-6, and TNF- α . Osteoporosis and psychological stress occur via different mechanisms and pathways involving RANKL (Stoppe and Dören, 2002; Kelly et al., 2019). The anti-RANKL treatment inhibited the NF- κ B pathway, increased the proportion of anti-inflammatory and non-cytotoxic M2 macrophages (Hamoudi et al., 2019).

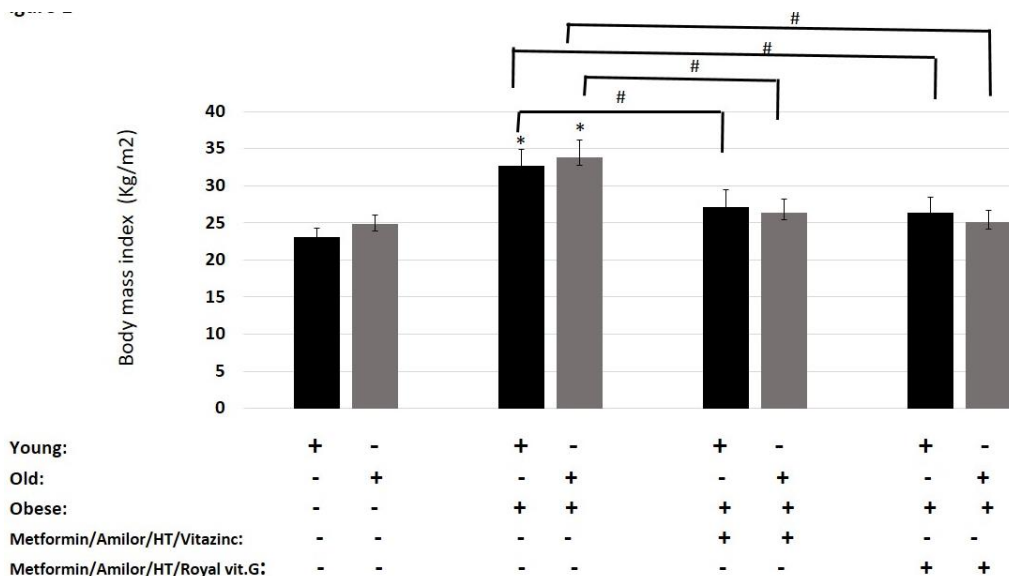


Fig.1. Effects of obesity and combined therapies on body mass index.

Hypoxia-inducible factor (HIF) is a transcription factor that plays an essential role in the cellular response to low oxygen and inflamed tissues that are often hypoxic. HIF helps immune cells adapt under normoxia during inflammation. HIF is implicated in multiple inflammatory diseases (McGettrick et al., 2020).

Vitazinc is a commonly prescribed multivitamin and minerals. It includes a combination of the mineral zinc in addition to components of vitamin B complex e.g. thiamine, riboflavin, niacin, vitamin B6, vitamin B12, folic acid, and pantothenic acid. Royal vitamin G capsules are more comprehensive in composition than Vitazinc. Royal vitamin G capsules include the same components of Vitazinc in addition to biotin, calcium, calcium pantothenate, copper,

dimethylaminoethanol bitartrate, ginseng, iron, lysine, manganese, nicotinamide, phosphorus, pollen, potassium, Royal Jelly, soya lecithin, and vitamin A. Based on its more ingredients, the therapeutic benefits of Royal vitamin G are expected to be broader in spectrum than Vitazinc.

Metformin is a biguanide drug that has been used to treat type 2 diabetes mellitus for more than 60 years. The United Kingdom Prospective Diabetic Study has shown Metformin to improve mortality rates in diabetes patients, and recent studies suggest Metformin has additional effects in treating cancer, obesity, nonalcoholic fatty liver disease, polycystic ovary syndrome, and metabolic syndrome. Metformin has also been shown to alleviate weight gain associated with antipsychotic medication (Zhou et al., 2018).

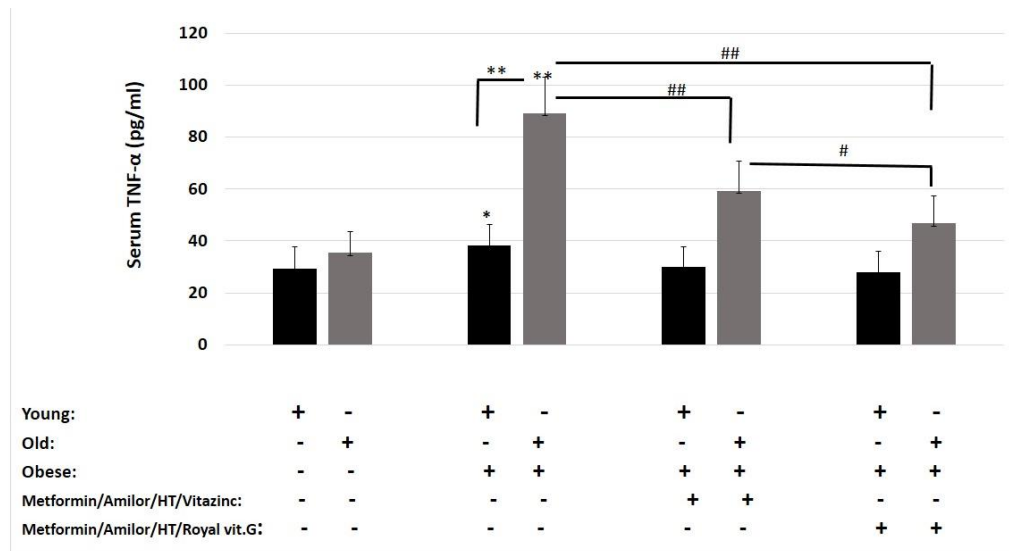


Fig.2. Effects of obesity and combined therapies on serum TNF-α (pg/ml).

A combination of Hydrochlorothiazide (diuretic) and Amiloride (potassium-sparing diuretic) was reported for effectively treating hypertension in obese subjects (Daniels et al., 1987). Moreover, two months of consumption of Royal Jelly was effective in reducing the severity of premenstrual tension syndrome (Taavoni et al., 2014). Vitamin D deficiency was reported as an important cause of unpleasant symptoms of premenstrual tension syndrome (Fatemi et al., 2019). A meta-analysis supported the use of calcium, vitamin B6, ginkgo, magnesium pyrrolidone, saffron, and vitamin E for treating premenstrual tension syndrome (Whelan et al., 2009).

In this study, we aimed at investigating the effects of obesity and combining Amiloride, Hydrochlorothiazide, Metformin, caloric restriction and walking exercise for six months on psychosomatic features of premenstrual tension syndrome

e.g. edema, anxiety, and fatigue. We also investigated the association of the biochemical mediators TNF-α, RANKL, HIF-α, and premenstrual tension syndrome.

Patients and methods

Study design

Our study is a case-control study that enrolled 60 obese women having premenstrual tension syndrome (depending on availability) who were categorized into younger age group (18-39 years) and older age group (40 -48 years) in comparison to 20 age-matched non-obese healthy women (10 young non-obese women younger than 40 years and 10 old non-obese women at or more than 40 years). This study preferred age grouping via using 40 years as a cut-off value (younger than 40 years versus older than 40 years). Blood samples were drawn from antecubital veins for biochemical assays at the end of the experimental

study. A prior ethical committee approval and informed patients' consent were taken From Faculty of Medicine, Tanta University, Egypt. Participation in the study was optional and participants had the right to withdraw at any time.

Inclusion criteria

- Women having premenstrual tension syndrome.
- Willingness to participate in the study.

Exclusion criteria

- Other psychiatric diseases.
- Critical illness

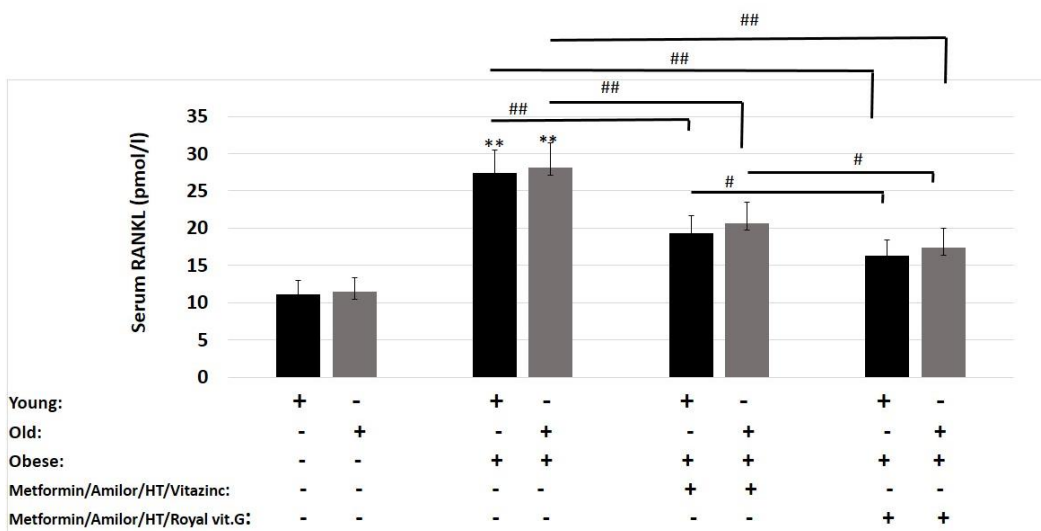


Fig.3. Effects of obesity and combined therapies on serum RANKL (pmol/l).

Pharmacological intervention:

All subjects maintained six months of caloric restriction diet in addition to half an hour of regular walking exercise a day and Moduretic intake (Amiloride hydrochloride/ Hydrochlorthiazide 5/50 mg) one week before menstruation onset for 6 consecutive cycles. Moreover, all subjects underwent six months of administration of Metformin combined with either Vitazinc capsules (comprising zinc gluconate, vitamin A, vitamin E components) or Royal vitamin G capsules.

Women's assessment and investigated parameters

Body mass index, TNF- α, HIF- α, and receptor activator of nuclear factor kappa

B (RANKL) were evaluated for all subjects.

In addition, psychosomatic manifestations were also evaluated before and after given treatments in the healthy control group and also in women having premenstrual tension syndrome. Assessed psychosomatic features included: edema, anxiety, and fatigue.

Body mass index calculation

That was done using the formula: Body mass index equals: Weight/ Length²

Assessment of psychosomatic manifestations

Anxiety assessment was done using Hamilton Anxiety Rating Scale as previously described (Hu et al., 2018).

Fatigue was rated in all subjects using the fatigue severity scale as previously reported (Ortelli et al., 2021). Edema was graded into none, mild, moderate, and severe edema that were given the rates 0,

1, 2, and 3, respectively. The percentage number of cases having psychosomatic manifestations was illustrated before and after combined therapies and presented as mean± SD.

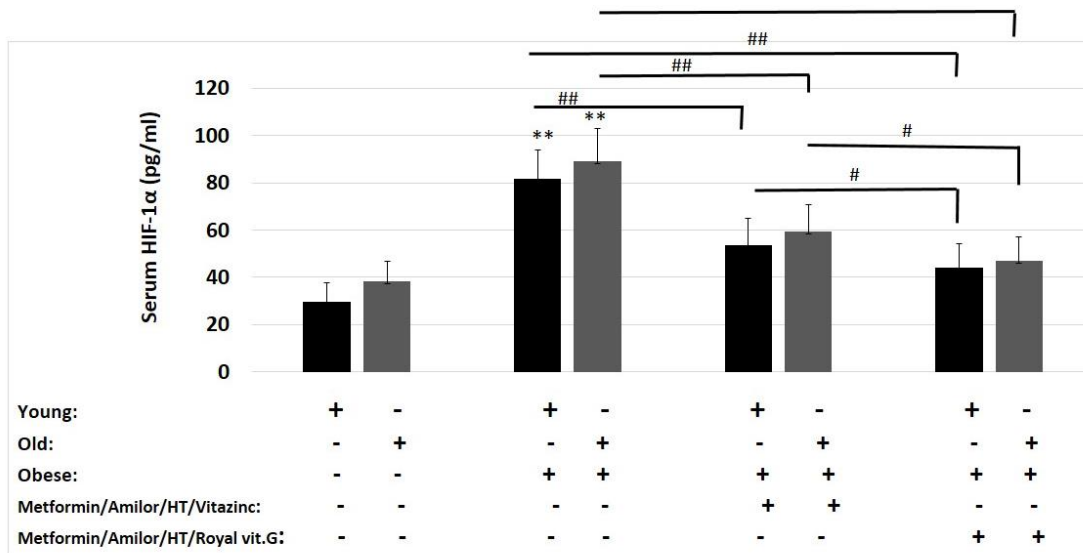


Fig.4. Effects of obesity and combined therapies on serum HIF-1α (pg/ml)

Serum HIF-1-α was significantly higher in obese women (in both age groups, p<0.01) compared to their controls (non-obese women of the same age group) and in older healthy women than in young healthy women (figure 4). Moreover, our data confirmed that combined treatment using (Metformin, Amiloride/ Hydrochlorthiazide, Vitazinc, caloric restriction diet, and half an hour of walking exercise a day) significantly decreased serum HIF-1-α to near normal values. Combined therapy-induced decrease in serum HIF-1-α was associated with a significant improvement of psychosomatic features (p<0.001) (fatigue, edema, and anxiety) (figures 5A-C). In addition, combined treatment using

(Metformin, Amiloride/ Hydrochlorthiazide, Royal vitamin G, caloric restriction diet, and half hour of walking exercise a day) significantly decreased serum HIF-1-α to near normal values. Combined therapy using Royal vitamin G decreased serum HIF-1α more significantly than combined treatment using Vitazinc (p<0.05).

Serum TNF-α assay

That was done using the Human TNF-alpha ELISA Test Kit (Genzyme, Cambridge, MA, USA) according to the manufacturer’s instructions.

Briefly, human recombinant TNF-α, used as a standard in this assay, was serially diluted from 800 to 12 pg/ml. A 96-well

microtiter plate coated with mouse monoclonal antibody specific for human TNF- α was incubated overnight. Standard amounts of human recombinant TNF- α and serum samples were added in duplicate. Afterward, the second antibody (rabbit anti-human TNF- α polyclonal antibody) and the third antibody (biotin-conjugated goat anti-rabbit IgG) were sequentially applied. Streptavidin-conjugated peroxidase was distributed into each well and a substrate reagent was added. The absorbance was measured at 492 nm using a multimode microplate reader (BioTek, USA). The results were expressed in pg/ml (Da-Cruz et al., 1996).

Serum RANKL assay

Serum RANKL was assayed in all subjects using a commercial ELISA (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions and as previously reported (Began et al., 2017). Briefly, plasma samples were loaded into microplates pre-coated with specific monoclonal RANKL and OPG antibodies. Standards and samples were bound by the immobilized antibody. After washing to remove unbound material, an enzyme-linked polyclonal antibody specific for RANKL and OPG was added to the wells. After a second wash with buffer, the substrate solution was added and incubated for 30 min while being protected from light. The reactions were stopped. The optical density of the color in each well was measured at 450 nm.

HIF-1 α assay

HIF-1 α was measured by enzyme-linked immunosorbent assay (Elabscience human ELISA kit, UK).

Data analysis and statistics

All data were statistically analyzed using SPSS software (version 13.00; SPSS Inc., Chicago, Illinois, USA). Data were expressed as mean \pm SD and analyzed by using one-way ANOVA analysis of variance. P values significance (*p <0.05, ** indicates p < 0.01 and *** indicates p < 0.001). #, # # and # # # indicate significance differences among different treatment conditions within the same group (# p <0.05, # # indicates p < 0.01 and # # # indicates p < 0.001).

Results

Our study enrolled obese versus non-obese women of two different age groups (younger than 39 years and older than that) (figure 1).

The effects of obesity and combined therapies on serum TNF- α (pg/ml) and psychosomatic manifestations

Serum TNF- α was significantly higher in obese women having premenstrual tension syndrome (in both age groups, p <0.05) compared to their controls. Interestingly, serum TNF- α was significantly higher (p <0.05) in older obese women than in younger ones (figure 2).

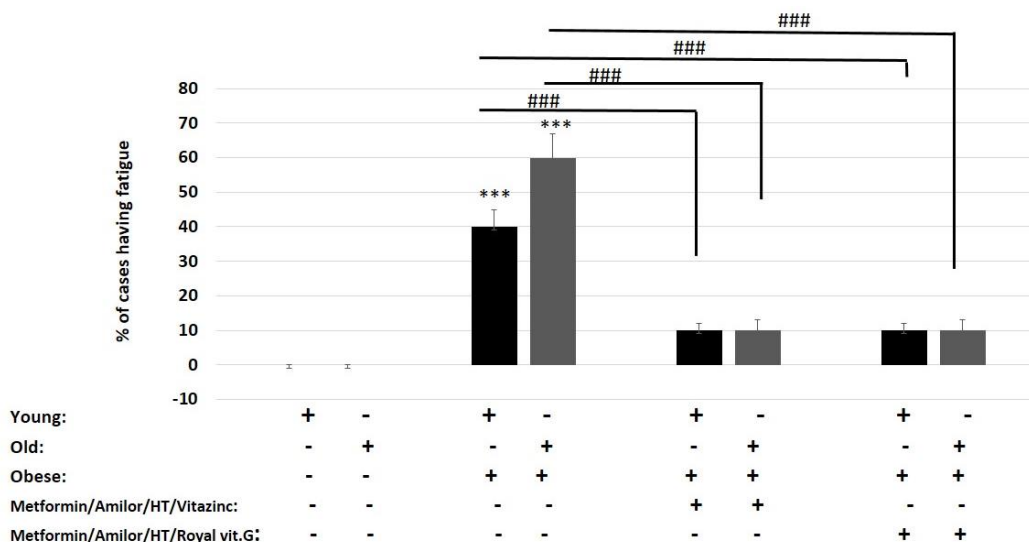


Fig.5A. Effects of obesity and combined therapies on psychosomatic manifestations of premenstrual tension syndrome. Obesity was associated with increased fatigue in both young and old obese women having premenstrual tension syndrome.

Later, our data revealed that both combined therapies using either (Vitazinc or Royal vitamin G), significantly decreased serum TNF- α to near normal values (figure 2). That was reflected on marked and significant ($p < 0.001$) improvement of psychosomatic features encountered in women having premenstrual tension syndrome (fatigue, edema, and anxiety) (figures 5A-C).

In addition, combined treatment using (Metformin, Amiloride/ Hydrochlorthiazide , Royal vitamin G, caloric restriction diet, and half an hour of walking exercise a day) significantly decreased serum TNF- α to near normal values (figure 2). That was reflected in marked and significant improvement of psychosomatic features (fatigue, edema, and anxiety) ($p < 0.001$) (figures 5A-C). Therapeutic benefits after combined therapy using Royal vitamin G caused a more significant decrease in serum TNF- α

($p < 0.05$) than combined therapy using Vitazinc (Metformin, Amiloride/ Hydrochlorthiazide, Vitazinc, caloric restriction diet, and half an hour of walking exercise per day).

Effects of obesity and combined therapies on serum RANKL (pmol/l) and psychosomatic manifestations

Our data revealed that serum RANKL was significantly higher in obese women (in both age groups, $p < 0.01$) compared to their controls.

Moreover, our data confirmed that both combined therapies using either (Vitazinc or Royal vitamin G), significantly decreased serum RANKL to near normal values (figure 3). That was reflected on marked and significant ($p < 0.001$) improvement of psychosomatic features encountered in women having premenstrual tension syndrome (fatigue, edema, and anxiety) (figures 5A-C).

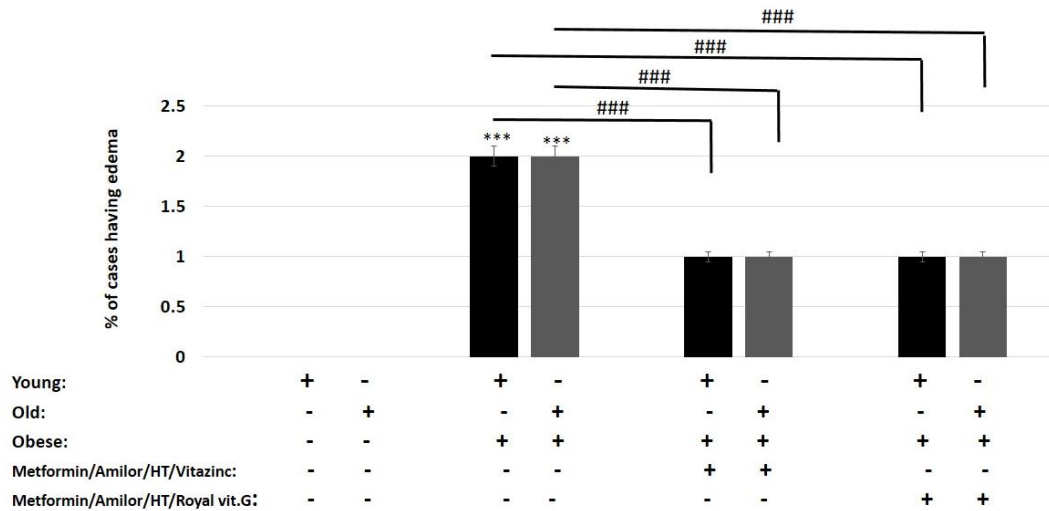


Fig. 5B. Obesity was associated with increased edema in both young and old obese women having premenstrual tension syndrome.

Effects of obesity and combined therapies on serum HIF-1α (pg/ml) and psychosomatic manifestations

Serum HIF-1-α was significantly higher in obese women (in both age groups, p<0.01) compared to their controls and in older healthy women than in young healthy women. Moreover, our data confirmed that both combined therapies using either (Vitazinc or Royal vitamin G), significantly decreased serum HIF-1 to near normal values (figure 4). That was reflected on marked and significant (p<0.001) improvement of psychosomatic features encountered in women having premenstrual tension syndrome (fatigue, edema, and anxiety) (figures 5A-C).

The magnitude of improvement of psychosomatic manifestations of women having premenstrual tension syndrome after combined therapies

Fatigue increased significantly (p<0.001) in obese subjects of both age groups compared to healthy controls (figure 5A). Both combined therapies using either (Vitazinc or Royal vitamin G), significantly decreased fatigue occurrence, with insignificant differences between Vitazinc-containing combined therapy and Royal vitamin G-containing combined therapy (figure 5A).

Edema increased significantly (p<0.001) in obese subjects of both age groups (figure 5C). Both combined therapies using either (Vitazinc or Royal vitamin G), significantly decreased edema occurrence, and with insignificant differences, between edema Vitazinc-containing combined therapy and Royal vitamin G-containing combined therapy (figure 5C).

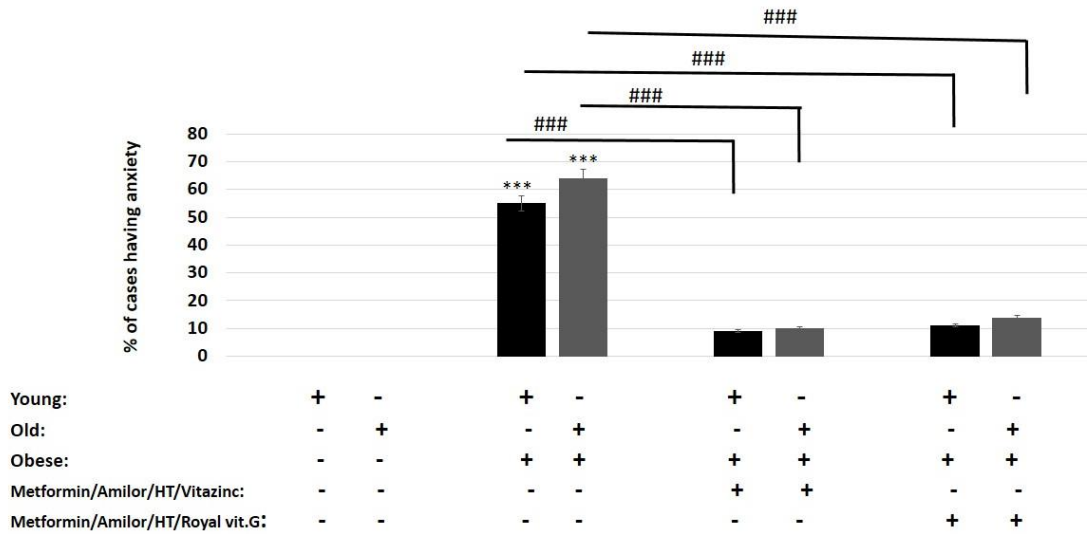


Fig. 5C. Obesity was associated with increased anxiety in both young and old obese women having premenstrual tension syndrome.

Anxiety increased significantly ($p < 0.001$) in obese subjects of both age groups (figure 5C). Both combined therapies using either (Vitazinc or Royal vitamin G), significantly decreased anxiety occurrence, with insignificant differences between Vitazinc-containing combined therapy and Royal vitamin G-containing combined therapy (figure 5C).

Discussion

Weight gain and obesity are significantly associated criteria that may exaggerate the psychosomatic manifestations associated with premenstrual tension syndrome in women. In a recent study, reported symptoms of premenstrual tension syndrome (in 91.7% of the investigated women) were irritability (73.33%) and physical symptoms, including swelling (65%), and anxiety (58.3%). Edema was detected in

many body regions e.g. facial, umbilical, pubic, epigastric, mammary, the mid-third of the arms, distal forearm, in both thighs, and the mid-third of the legs (Tacani et al., 2015). Our data agreed with this report and edema is present in females having premenstrual tension syndrome. Moreover, increased body mass index was significant (compared to healthy control groups, $p < 0.05$) in the two investigated age groups.

Our data revealed that both combined treatments using either (Vitazinc or Royal vitamin G), together with a caloric restriction diet, and half an hour of walking exercise a day, significantly decreased body mass index to near normal values, this was associated with a significant improvement in the investigated psychosomatic features (fatigue, edema, and anxiety).

Our data revealed also that the inflammatory marker (TNF- α) was significantly higher ($p < 0.01$) in obese women having premenstrual tension syndrome. This agrees with the study by Azizieh et al. who reported that serum levels of pro-inflammatory cytokines (IL-8 and TNF- α) were significantly higher in premenstrual tension syndrome compared to subjects who reported no symptoms. The proinflammatory: anti-inflammatory cytokine ratios showed increased pro-inflammatory cytokine. There is a possible role of pro-inflammatory cytokines as a contributing factor in self-reported symptoms related to premenstrual tension syndrome (Azizieh et al., 2017).

Our data revealed that both combined treatments using either (Vitazinc or Royal vitamin G), with a caloric restriction diet, and half an hour of walking exercise a day, significantly decreased the inflammatory marker (serum TNF- α) to near normal values. This was associated with a significant improvement in the investigated psychosomatic features (fatigue, edema, and anxiety).

During menopause, there is increased production of pro-inflammatory factors particularly inflammatory cytokines e.g. IL-1 IL-6, and TNF- α , as well as estrogen's regulatory role in osteoclast receptor signaling, including RANKL. Estrogen replacement therapy reverses such effects (Kelly et al., 2019). This is also in exact agreement with our

findings where serum TNF- α was higher in older healthy women compared to younger ones.

Interestingly, Denosumab is a monoclonal antibody to RANKL, a ligand expressed by osteoblasts that is necessary for the differentiation of osteoclasts. Denosumab sequesters RANKL and prevents its interaction with osteoclastic RANK, mimicking the natural function of osteoprotegerin resulting in decreased formation of osteoclasts i.e. decreased bone resorption. Treatment with denosumab also decreases the risks of hip, vertebral, and non-vertebral fractures. The effects of denosumab treatment on mental health need further research investigations. However, Suzuki et al. demonstrated that denosumab treatment altered magnesium levels that impact mental health.

Interestingly, activation of the sympathetic nervous system, commonly associated with chronic psychological stress, affects the peripheral expression of RANKL on osteoblasts and T cells (Kelly et al., 2019).

HIF-1 α and hypoxia play a crucial role in dendritic cells activation in inflammatory states, marked increases in expression of costimulatory molecules, proinflammatory cytokine synthesis, and induction of allogeneic lymphocyte proliferation, which is highly dependent on glycolysis even in the presence of oxygen. HIF-1 α followed the same

pathway to TNF- α and RANKL in women having premenstrual tension syndrome (Jantsch et al., 2008).

Our data revealed that HIF-1 α increased with obesity and decreased with combined treatment using either Vitazinc or Royal vitamin G with a more significant decrease in HIF-1 α when using Royal vitamin G than when using Vitazinc as a component of combined therapy in the studied two different age groups. This may be attributed to the more enormous constituents of Royal vitamin G capsules than Vitazinc.

Fatigue increased significantly ($p < 0.001$) in obese subjects of both age groups. Combined treatments including Vitazinc or Royal vitamin G caused a highly significant decrease ($p < 0.001$) in fatigue occurrence with insignificant differences between the two combined treatment regimens. The magnitude of reduction in fatigue was dramatic and massive upon using either combined treatments for the scheduled duration of time. No single reduction in any of the tested inflammatory markers can explain the massive reduction in fatigue. It is likely that a combined effect of all the investigated parameters collectively participated in reducing fatigue.

Edema increased significantly ($p < 0.001$) in obese subjects of both age groups. Combined treatments using

(Metformin, Amiloride/
Hydrochlorthiazide, caloric restriction diet, and half hour of walking exercise a day) with either Vitazinc or Royal vitamin G caused significant decreases in Edema occurrence. No significant difference was observed between Vitazinc-containing combined therapy and Royal vitamin G-containing combined therapy. The magnitude of reduction in Edema was dramatic and massive upon using either combined treatments for the scheduled duration of time. No single reduction in any of the tested inflammatory markers can explain the massive reduction in Edema. It is likely that a combined effect of all the investigated parameters collectively participated in reducing Edema in women having premenstrual tension syndrome.

Anxiety increased significantly ($p < 0.001$) in obese subjects of both age groups. Combined treatment using (Metformin, Amiloride/
Hydrochlorthiazide, caloric restriction diet, and half hour of walking exercise a day) with either Vitazinc or Royal vita G caused significant decreases in anxiety occurrence with no significant difference among both combined therapies. The magnitude of anxiety reduction was dramatic and massive upon using either combined treatments for the scheduled duration of time. No single reduction in any of the tested inflammatory markers can explain the massive anxiety reduction.

It is likely that a combined effect of all the investigated parameters collectively participated in reducing anxiety severity.

Fennel, a natural remedy of prophetic medicine exerts excellent psychiatric effects that combat a lot of cardinal manifestations of premenstrual tension syndrome and is strongly recommended for treating psychosomatic manifestations of premenstrual tension syndrome (Badgujar et al., 2014).

Conclusion

Our study concluded that premenstrual tension syndrome has biochemical and inflammatory bases that strongly increase its associated psychosomatic features. Using combined therapies (Amiloride/Hydrochlorothiazide, Metformin, caloric restriction, and exercise) with either Vitazinc or Royal vitamin G helped reducing obesity and psychosomatic manifestations.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

The authors are grateful to the deanship of scientific research and the medical research centre for kindly supporting this research work.

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