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Evaluation of Hyperbaric Oxygen Therapy for Diabetic Wounds and Transcutaneous Oximetry as a Predictor of Wound Healing: A prospective Study at Prana HBOT Center Mumbai

Manoj Gupta

Abstract

Background: It is general trend amongst patients with diabetes that usually they are at risk for developing foot ulcers irrespective of age, gender, symptoms, or adequacy of glycemic control. HBO therapy is currently approved for a variety of tissue healing and other applications. Transcutaneous Oximetry [TCOM] is a simple, reliable noninvasive technique for the objective assessment of wound perfusion and oxygenation. Its definitive role in predicting the wound healing has not yet been proven. Objective: We undertook a prospective study to evaluate the role of Hyper Baric Oxygen Therapy in healing of diabetic wounds and to determine whether Transcutaneous Oximetry can predicts healing. Study Design: A prospective study was performed to realize the aim and objectives of this study. Place of Study: The study was carried out at the Prana HBO Centre, which is owned by the Investigator and located in the Northern parts of Mumbai, in India. Methods: The center has one Sechrist Monoplace hyperbaric chamber and a TCOM machine with 3 electrodes. The oxygen gas supply is from oxygen cylinders of 7000 liters' capacity each. The data was collected from all the patient files, which are stored at the center. Transcutaneous Oximetry monitoring system was utilized for measuring tissue oxygenation (TcPO₂) in all the patients of both groups. Observation and Discussion: There was 68% reduction in wound area (mean baseline area of 22 cm² decreased to 7 cm² at 30 day of therapy, as per there mean value in group HT by using paired t test, there is highly significantly improvement in wound with p value 0.00001. Hyperbaric oxygen therapy increases the chances of healing of ulcers by decreasing exudates and very well promotes granulation tissue, as well the wound size decreases and wound tissue type and healing is improvised. Conclusion: Hyperbaric Oxygen Therapy definitely has an adjunctive play in management of non healing ulcers usually in diabetic patients.

Keywords: Transcutaneous Oximetry; Hyperbaric Oxygen Therapy; Ulcers.

Introduction

Diabetics succumb to plenty of foot disorders, including, neuropathy, infection, Charcot arthropathy, and peripheral arterial disease, amongst them foot ulcers are relatively most common and characteristic diabetic foot wound, and they are significant source of morbidity and disability [1]. By definition Ulcers are defined as any break in the cutaneous barrier that usually extends through the full thickness of the dermis.

It is general trend amongst patients with diabetes that usually they are at risk for developing foot ulcers irrespective of age, gender, symptoms, or adequacy of glycemic control. Multiple Factors associated with an increased risk of foot ulcers in patients with diabetes shall include neuropathy, foot deformity, limit joint mobility, trauma, ischemia, male sex, and previous history of ulceration [2]. Screening tests in diabetics can help identify patients at increased risk of diabetic foot ulceration and include neuropathy symptoms.
and disability scoring indexes, measurement of vibration perception threshold and peak plantar foot pressures [3].

Patients with diabetes at increased risk for foot ulceration may show some benefit from prophylactic interventions which also include education, prescription footwear, intensive podiatric care, and evaluation for surgical interventions [4].

Hyperbaric Oxygen therapy (HBOT) is a systemic treatment option; wherein a patient breathes pure oxygen at pressures higher than normal atmospheric pressure at sea level in a hyperbaric chamber. (one ATM is equal to around fourteen pounds per square inch (PSI), 1 kilogram per square centimeter). Therapeutic effects of hyperbaric oxygen therapy are due to increase in dissolved oxygen in plasma and tissue oxygen delivery. It is proved that Oxygen is a has an important role in the physiology of wound healing. HBO therapy is a useful adjunct in the treatment of diabetic foot ulcers by it heals to increase tissue oxygen tensions to the levels that shall promote wound healing and limit edema, and shall also destroy certain anaerobic bacteria, it augmenting neo-vascularization, stimulates fibroblast proliferation and differentiation, increases collagen formation as well stimulate leukocyte microbial killing [5,6]. HBOT therapy is currently approved for a variety of tissue healing and other applications.

Transcutaneous Oximetry [TCOM] is a simple, reliable noninvasive technique for the objective assessment of wound perfusion and oxygenation. The definite role of transcutaneous Oximetry in analyzing a wound healing had not yet been proved clearly. We undertook a prospective study to evaluate the role of Hyper Baric Oxygen Therapy in healing of diabetic wounds and to determine whether Transcutaneous Oximetry can predicts healing [7].

**Material and Methods**

**Study Design**

A prospective study was performed to realize the aim and objectives of this study.

**Study setting**

The study was carried out at the Prana HBO Centre, which is owned by the Investigator and located in the Northern parts of Mumbai, in India. The center has one Sechrist Monoplace hyperbaric chamber and a TCOM machine with 3 electrodes. The oxygen gas supply is from oxygen cylinders of 7000 liters’ capacity each. The center has all the requisite certifications and registrations as required by the local authority in Mumbai. The data was collected from all the patient files, which are stored at the center. Transcutaneous Oximetry monitoring system was utilized for measuring tissue oxygenation (TcPO$_2$) in all the patients of both groups. Measurements were recorded on non inflamed skin 1 cm proximal to the upper margin of ulcer. TcPO$_2$ findings were recorded on zero, tenth, twentieth, and thirtieth day. TcPO$_2$ was used and the findings were calculated by an electrochemical transducer, and it remain attached to skin and use of adhesive ring and contact liquid was used. The measuring site was cleaned carefully by a disinfectant (spirit). By analyzing and measuring the oxygen reduction current with the help of measuring cell it was concluded for skin oxygen partial pressure. TCOM data was also collected from the Centre register which is maintained separately. The Investigator is the medical practitioner working in this center and the physician who consulted the patients. The study is limited to the patients who were seen at the Centre during two years. Written informed consent was obtained from all the patients and record maintained.

**Inclusion criterion**

Patients referred from surgical specialties to the hyperbaric oxygen therapy center Prana Mumbai, with the complaint of non healing ulcer, were included in the study. Patients in the age group of 20–68 years of either sex with diabetic non healing ulcer, despite receiving conventional therapy, were included in the study.

**Exclusion Criterion**

The study excluded all those patients who were consulted by the Investigator but were not treated with HBO, nor they were evaluated for HBO (e.g. by means of TCOM studies) and Patients who were not willing for HBOT, those with active upper respiratory tract infection, with any active lung pathology or pregnancy were excluded from the study.

**Statistical Analysis**

All the data was collected from patient and the findings noted in register, which is manually maintained at the center. The primary outcome variable was the wound size. The collected data
was directly captured in an MS excel spread sheet for analysis. Factors associated with following the approved protocols, association was determined by calculating the descriptive statistics, correlation, with 95% confidence intervals. The paired and unpaired t test was used to determine statistical significance between the two groups and pre and post exposure. A significance level of 0.05 was used for all these tests.

**Ethics review**

This study was performed within the scope of international ethical guidelines and legislation. Ethics review and approval was provided by Stellenbosch University (number: U16/06/015) and the ethics committee of the Hyperbaric Society in India.

**Observations**

In the study total more than 67 cases were recruited and ended up with final 56 number of patient who fulfilled all the inclusion criteria for the study. Total 56 patients completed the study period and no patient was excluded during the study analysis. The demographic profile was comparable in the two groups of hyperbaric therapy and conventional treatment groups. Classification of diabetic foot ulceration was Adapted from Wagner [8].

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<tr>
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<tr>
<td>1</td>
<td>Superficial ulcer with subcutaneous involvement</td>
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<tr>
<td>2</td>
<td>Deep ulcer with tendon or joint involvement</td>
</tr>
<tr>
<td>3</td>
<td>Deep ulcer with bone involvement</td>
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<tr>
<td>4</td>
<td>Wet or dry gangrene (forefoot), without cellulites</td>
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<tr>
<td>5</td>
<td>Generalized (whole foot) gangrene</td>
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In reference to the co morbidities of the patients, refer to table no 2, wherein 15 patients with diabetes mellitus and hypertension were smokers. Common sites of wound were leg 13, forefoot 10, mid foot 14, great toe 10, other toe 4 and other sites were around 5 no of cases.

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<td>52±11.2</td>
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<td>24/04</td>
<td>22/06</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Co morbidities</th>
<th>Group HT</th>
<th>Group CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Hypertension</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Varicose vein</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Vascular insufficiency</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Smoking</td>
<td>14</td>
<td>15</td>
</tr>
</tbody>
</table>

There was 68% reduction in wound area (mean baseline area of 22 cm² decreased to 7 cm² at 30 day of therapy) as per there mean value in group HT by using paired t test, there is highly significantly improvement in wound with p value 0.00001. There was mild change in wound area which shows 8% decrease in wound area (mean baseline area of 20.5 cm² changed to 18.9 cm² at 30 day of therapy, by using paired t test, there was no significant improvement in wound with p value 0.1938 in group CT. (Table 2).

![Time duration wise peri wound TcPO2 values](image)
Table 3: Comparison of wound tissue score from baseline at different time intervals of therapy

<table>
<thead>
<tr>
<th>Variables</th>
<th>Improvement in wound score (n)</th>
<th>No change in wound score (n)</th>
<th>Deterioration in wound score (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group HT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 28)</td>
<td>After 10 days</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>After 20 days</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>After 30 days</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td><strong>Group CT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 28)</td>
<td>After 10 days</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>After 20 days</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>After 30 days</td>
<td>7</td>
<td>19</td>
</tr>
</tbody>
</table>

A significant improvement in wound score was observed in group HT as compared to group CT after 30 days of treatment. Refer table No 3. Significant improvement in wound score was observed after every 10 sittings of HBOT in group HT, after 30 days of treatment 20 patients showed improvement in group HT, whereas no change was observed in 6 patient of HT group and there had been deterioration in wound score in 2 cases of HT group. As compared to group HT, patient who received only CT therapy also showed improvement in 7 patients, whereas no change in 19 patient of CT group, 2 patient of group Ct there had been deterioration in wound score. (Table 3).

As per Table 4, a positive correlation was found between TcPO$_2$ value and various markers of wound healing, such as decrease in area of the wound, decrease in exudates amount, and improvement in wound score. In observation it was found to be negative correlation amongst amputation rate and the value of TcPO$_2$. Peri wound TcPO$_2$ values were monitored at regular intervals in both the groups and a positive correlation was found between TcPO$_2$ values and various markers of wound healing, such as decrease in area of the wound ($P = 0.000567$), decrease in exudates amount ($P = 0.000021$), and improvement in wound score ($P = 0.0047$). A negative correlation of (-0.56) was found between TcPO$_2$ values and amputation rate ($P = 0.000972$). Higher the peri wound TcPO$_2$ levels more are the chances of wound healing. It was observed that correlations individually were very significant in group of HT whereas it was not significant in group CT.

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Discussion

In our study it is very clear that hyperbaric oxygen therapy increases the chances of healing of ulcers by decreasing exudates and very well promote granulation tissue, as well the wound size decreases and wound tissue type and healing is improvised. It is also very clear from our observation that TcPO$_2$ values and various other parameters of wound healing taken into consideration in this study such as decrease in area of the wound, decrease in exudates amount and improvement in

Table 4: Correlation of peri wound TcPO$_2$ and various parameters of wound healing

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group HT (n = 28)</th>
<th>Group CT (n = 28)</th>
<th>Combined Group (n = 56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improvement in tissue grading</td>
<td>0.64</td>
<td>0.000123</td>
<td>0.3112</td>
</tr>
<tr>
<td>(decrease in wound score)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement in exudates</td>
<td>0.768</td>
<td>0.000001</td>
<td>0.2861</td>
</tr>
<tr>
<td>(decrease in exudates)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in wound size</td>
<td>0.7948</td>
<td>0.000001</td>
<td>0.2951</td>
</tr>
<tr>
<td>(decrease in wound area)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amputation rate</td>
<td>-0.5837</td>
<td>0.000566</td>
<td>-0.2349</td>
</tr>
</tbody>
</table>

![Fig. 2: A diabetic patient was accomplished by utilizing conventional modalities including a course of hyperbaric oxygen therapy.](image)
wound score. It was also seen in our findings that a negative correlation was seen between TcPO$_2$ values and amputation rate.

Findings of other authors like Oriani et al. [9] and Baroni et al. [10], who reported that better healing changes and least amputation rate was appreciated after hyperbaric oxygen therapy as compared to conventional therapy in diabetic patient with chronic non healing ulcers, this findings are in concurrent agreement with our findings and study and shows a positive effects of adjunctive of hyperbaric oxygen therapy. In addition, as per Jain KK [11] et al. Hyper Baric Oxygen Therapy has bactericidal and bacteriostatic effects on both aerobic and anaerobic bacteria through the action of the super oxide enzyme, which acts more rapidly at high oxygen tensions (30 to 40 mm Hg).

As per Brakora MJ [12] et al. Hyperbaric Oxygen Therapy has also been shown to have synergistic effects with aminoglycosides, trimethoprim, nitrofurantoin, and sulfisoxazole. Furthermore, hyperoxic vasoconstriction that takes place during Hyper baric Oxygen Therapy reduces capillary pressure and increases vascular permeability. The resulting decrease in transcapillary fluid transfer increases extravascular fluid resorption, which reduces lower extremity edema [13]. Niinikoski JH et al. [14] In his animal studies, demonstrated that wound healing was an oxygen dependent process by measuring transcutaneous oxygen pressure TcP02. By employing the same technique, Sheffield [15] demonstrated that chronic tissue hypoxia could be corrected by HBOT. As per Wattel F [16] et al. hyperbaric oxygen therapy may be added as to conventional treatment of diabetic foot ulcers, if it is clearly that peri wound TcPO$_2$ in 2.5 ATA HBO is over 200 mm Hg.

Complication during treatment, none of the patients suffered a serious complication from HBO, such as a pneumothorax, or seizure etc. However, some minor side-effects were experienced like ear pain in the chamber most likely as a result of mild barotrauma of the ears. However, Barotrauma wasn’t noted as a complication for any of these patients. One patient suffered hypoglycemia during a treatment, while eleven patients suffered visual changes related to the HBO.

Conclusion

To conclude Hyperbaric Oxygen Therapy definitely has an adjunctive play in management of non healing ulcers usually in diabetic patients. It is well settled by the study that the complicated wound require immediate attention with multidisciplinary approach, aggressively which include administration of hyperbaric oxygen therapy. However conventional treatment and approach is also equally required to decrease the amputation rate and to increase the well outcome of the patients. It is clear from the study that TcPO$_2$ values shall be useful in predicting the response of hyperbaric oxygen therapy and definitely have a positive correlation with regards to wound healing. The results of this study will definitely contribute to evidence-based decision making on the use of Hyperbaric Oxygen Therapy as an adjunctive therapy in patients with a diabetic foot ulcer.

Conflict of Interest: The author declares no conflict of interest for this study.

Acknowledgement

Author acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript.

Reference


Patients with Type-2 Diabetes Mellitus and Hypertension: A Cross-sectional, Observational, Pan-India Study

PG Talwalkar¹, Vaishali Deshmukh², M.C. Deepak³, Dinesh Agrawal⁴

Abstract

Background: The objective of this clinico-epidemiological, Pan-India study was to evaluate the prevalence of vitamin D (vitD) deficiency in Indian patients with type-2 diabetes mellitus (T2DM), hypertension (HT) and both T2DM and HT and to understand their management practices.

Methods: Adults with a diagnosis of T2DM, HT or both were enrolled across 29 sites in India. All the patient-related data were extracted from medical records. VitD insufficiency and deficiency was defined as serum 25(OH)D levels 21-29 ng/ml and ≤20 ng/ml, respectively.

Results: A total of 1501 (99.5%) patients completed the study (T2DM: 99.2%; HT: 99.6%; T2DM+HT: 99.8%). Mean age at diagnosis of vitD deficiency was 52.5±10.77 years. Prevalence of patients with low vitD levels (vitD deficiency [60.9%] and insufficiency [22.9%]) was 1257 (83.7%); 1231 (82%) were newly diagnosed cases. Prevalence of low vitD levels amongst patients with T2DM, HT and T2DM+HT was 84.2%, 82.6% and 84.5%, respectively. Out of 1257 patients, 84.8% received vitD supplementation. Preferred dose and route of administration was 60,000 IU (70.2%) and oral (79.6%). Preferred frequency of dose was once in a week (76.7%). VitD deficiency (26.9%), vitD insufficiency (34.5%), symptoms of vitD deficiency (10.4%) and co-morbid condition (1.8%) were the factors considered while prescribing vitD supplements.

Conclusion: Prevalence of vitD deficiency was higher in newly diagnosed cases compared to already diagnosed cases in all three cohorts. This study indicates the magnitude of overlap between vitD deficiency and T2DM or HT, and the need for routine screening for early diagnosis and effective management.

Keywords: Deficiency; hypertension; type-2 diabetes mellitus; insufficiency.

Introduction

Non-communicable disease continues to be an imperative public health problem in India, leading to substantial increase in mortality and morbidity. Among these, Type-2 diabetes mellitus (T2DM) and hypertension (HT) are increasing at an alarming rate [1].

Defects in pancreatic β-cell function, insulin sensitivity, and systemic inflammation are few of the contributing factors towards the development of T2DM. On the other hand, HT results due to the imbalance between vasoconstriction and vasodilation, favoring vasoconstriction. Genetic and epigenetic factors, including nutritional risk factors, plays a major role in the pathogenesis of these diseases.

Recently, an intersecting underlying pathology between T2DM and HT with vitamin D (vitD) insufficiency/deficiency has been noted [2-4]. The presence of vitD receptors on the pancreatic beta cells, adipose tissues and skeletal muscle cells indicates the function of vitD in the glucose metabolism [5-7]. Further, it has been propounded that altered vitD and calcium homeostasis may play a role in the development of T2DM [8]. VitD enhances the synthesis of insulin hormone and its release from the pancreatic beta cells. VitD may play
a functional role on glucose tolerance through its effects on insulin secretion and insulin sensitivity [9]. Furthermore, 25-hydroxyvitD (25(OH) D) inhibits the release of the pro-inflammatory cytokine TNFα, regulates the activity of NF-κB, and suppresses the expressions of TLR2 and TLR4 proteins and mRNA in human monocytes, reducing the release of cytokines. Thus, vitD may also function to reduce insulin resistance and the risk of diabetes by decreasing inflammatory responses [10,11]. Thus, patients with low vitD levels may be at higher risk of developing T2DM, worsening glycemic control, increasing serum lipid levels, thereby leading to a higher prevalence of cardiovascular disease. Chiu C and Audrey in a study in 126 healthy participants showed a direct relation between insulin sensitivity and 25(OH)D level and that vitD deficiency has a negative effect on pancreatic β-cell function [12].

Low vitD levels are also found to be associated with an increased risk of HT and cardiovascular events in population-based studies [13-15]. Studies suggest that vitD supplementation may decrease blood pressure in patients with T2DM, although conflicting results have been reported [16]. Vit D had an inhibitory effect on the synthesis of renin, by converting angiotensin I to II, or on the regulation of inflammation in laboratory animals [17,18]. VitD improves endothelial function by reducing vascular inflammation, regulating blood pressure, inhibiting proliferation of vascular smooth muscle cells, and antagonizing the formation of foam cells [19]. Further, antioxidant property of vitD complements the protective mechanisms on heart and vasculature. Sugden et al in their study showed that a single large dose of vitD2 (100,000 IU orally) improved blood pressure and endothelial function, a key surrogate marker of cardiovascular risk, at 8 weeks post dose, in patients with T2DM and baseline 25OHD levels below 50 nmol/l [20].

Owing to these multifarious implications on health, the epidemic of vitD deficiency in India is likely to contribute significantly to the enormous burden on the healthcare system of India. Further, the increasing prevalence of T2DM and HT, either alone or in co-existence, in a populated country like India, increases the peril of devastating complications and eventual mortality. Hence the objective of this study was to evaluate the prevalence of vitD deficiency in patients with T2DM, HT and both T2DM and HT, and to understand the current management practices in Indian real-world setting. Association between vitD levels and hypothyroidism, if any, was also ascertained in this study. Early detection and treatment of vitD deficiency in these disorders is imperative to achieve the treatment goals. To the best of our knowledge, this is the first of its kind study to evaluate the trend of co-occurrence of vitD deficiency and the management practices in Indian patients.

Materials and methods

Study design and Patient population

This cross-sectional, clinico-epidemiological, multicentric, PAN – India study was conducted across 29 centers, spanning different geographical sites in India, between June to September 2017. Adults (≥18 years) with a diagnosis of T2DM and/or HT (established [who were already receiving antidiabetic/antihypertensive therapies] or newly diagnosed cases [fasting plasma glucose (FPG) ≥ 126 mg/dL or glycated hemoglobin (HbA1c) ≥ 6.5% for T2DM and blood pressure (BP) ≥ 140/90 mmHg for HT], visiting physician for routine check-up were enrolled in this study. Patients on a fixed dose combination of calcium and vitD supplement or with a history of using drugs that may interfere with vitD metabolisms (eg: carbamazepine, phenobarbital, sodium valporate, gabapentin, isoniazid, corticosteroids, mineral oil and calcitonin) were excluded from the study.

Three cohorts were formed in this study based on the indication, viz., T2DM, HT, and T2DM+HT. Each cohort contained approximately equal number of patients recruited in a ratio of 1:1:1.

The study protocol was approved by local independent ethics committees. The study was conducted in accordance with the principles of Declaration of Helsinki, International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines, and Indian regulatory guidelines (Indian Council of Medical Research and Indian GCP guidelines). All patients provided written consent in the patient authorization form to participate in the study.

Data Collection

After obtaining necessary permissions from the hospital authorities, the patient data were collected from their hospital medical records. The patient data included demographics and anthropometric characteristics, medical/surgical/family history, lifestyle parameters, concomitant medications, physical examination, vital measurements, details of T2DM and HT, including duration of disease, medications (dose, regimen), latest glycemic indicator values (FPG, postprandial glucose [PPG], HbA1c). In addition to patient hospital records, the
blood sample was collected from each patient for serum 25(OH)D analysis.

**Study Definitions:**

**VitD:**
- VitD deficiency
  25 (OH) D: < 20 ng/mL
- VitD insufficiency
  25 (OH) D: 20–29 ng/mL
- VitD sufficiency
  25 (OH) D: ≥ 30 ng/mL

**Hypothyroidism:**
- Overt hypothyroidism
  TSH > 4.50 μIU/mL, fT4 < 0.8 ng/dL, and fT3 < 1.4 pg/mL.
- Subclinical hypothyroidism
  TSH > 4.50 μIU/mL, fT4 0.8-1.8 ng/dL, and fT3 1.4-4.4 pg/mL

**Obesity and Overweight**
- Overweight
  BMI: ≥ 23 and < 25 kg/m²
- Obese
  BMI: ≥ 25 kg/m²

**Study Outcome**

The primary outcome was the percentage of patients with VitD deficiency in those with T2DM, HT or T2DM and HT. Association between different categories of VitD levels (deficient, insufficient and sufficient) and hypothyroidism and the prevailing management practices, i.e. percentage of patients receiving VitD supplementation along with regimen (doses in IU, frequency and recommended duration) were also assessed in this study.

**Statistical Analysis**

Considering the lack of data on the prevalence of VitD deficiency in patients with T2DM or HT or both T2DM and HT, approximately 1500 patients, including 500 patients of T2DM, HT and T2DM+HT each were enrolled in the study, assuming 10% drop-out rate. Continuous variables were summarized descriptively. Categorical data was presented as frequencies and percentages. The association between different categories of VitD levels and hypothyroidism was derived by chi-square test at 5% level of significance. All statistical analyses were performed using SAS® version 9.4 (SAS Institute Inc, USA).

**Results**

Initial data was collected from 1508 patients (T2DM: 504; HT: 501; T2DM+HT: 503). Of these, 1501 (99.5%) completed the study (T2DM: 500 [99.2%]; HT: 499 [99.6%]; both T2DM and HT: 502 [99.8%]). Seven patients (T2DM: 4; HT: 2; T2DM+HT: 1) discontinued the study by withdrawing their consent.

**Baseline and Demographic Characteristics**

The mean±SD age of the overall population was 52.9±12.49 years. The mean ± SD age of the patients with T2DM, HT and both T2DM and HT was 50.7±12.18, 51.6±13.58, and 56.4±10.82 years, respectively. In the overall population, 792 (52.5%) were women and 716 (47.5%) were men. Baseline and demographics characteristics of patients from all the 3 cohorts are shown in Table 1.

From the total cohort, 1007 patients were reported to have T2DM. The mean duration of T2DM was 8.3±6.90 years; mean age at the time of diagnosis was 45.3±10.31 years. The HT was reported in 1004 patients. The mean duration of HT was 6.8±5.87 years; mean age at the time of diagnosis was 47.3±11.20 years. About 8.3% patients with T2DM and 5.9% patients with HT had complications due to their indications. The most common complication due to T2DM and HT were diabetic neuropathy (52.4%) and renal disease (52.5%), respectively.

Forty-two (2.8%) patients had known history of VitD deficiency (T2DM: 16 [3.2%]; HT: 12 [2.4%]; T2DM and HT: 14 [2.8%]). The mean duration of VitD deficiency was 1.9±4.46 years. The mean age at the diagnosis of VitD deficiency was 52.5±10.77 years. In total, the mean VitD level at the time of diagnosis was 16.9±12.78 ng/ml.

The serum 25 (OH)D levels reported from 24 patients at the time of diagnosis was 22.4±16.31 ng/ml in patients with T2DM (n=7), 18.8±13.10 ng/ml in patients with HT (n=8), and 10.9±7.03 ng/ml in patients with both T2DM and HT (n=9). Majority of the patients with known history of VitD deficiency were treated with cholecalciferol (T2DM: 16; HT: 12; T2DM+HT: 13); one patient with T2DM+HT was treated with calcitriol.
Prevalence of vitamin D deficiency

Overall prevalence of low vitD was found to be 83.7% (1257 out of 1501 patients; T2DM: 421 [84.2%]; HT: 412 [82.6%]; T2DM+HT: 424 [84.5%]). The remaining 244 patients (T2DM: 79; HT: 87; T2DM+HT: 78) had vitD sufficiency.

Already established versus newly diagnosed cases with low level of vitD in patients with T2DM, HT and combined condition of T2DM and HT are summarized in Table 2. The prevalence of patients with low level of vitD amongst newly diagnosed cases was 1231 (82.0%) compared to 26 (1.7%) in already diagnosed cases. Number of already and newly diagnosed cases with low level of vitD were comparable in each cohort (Table 2).

Vitamin D levels in patients with T2DM, HT and combined condition of T2DM and HT

The new and already diagnosed cases with different categories of vitD levels in patients with T2DM, HT, and T2DM+HT are summarized in Table 3. More than half (60.9%) of the patients were reported with vitD deficiency. The highest number of vitD deficient cases were from T2DM only category (64.0%), followed by combined T2DM and HT (61.6%) and HT only (57.1%). VitD insufficiency was highest in HT only category (25.5%), followed by combined condition of T2DM and HT (22.9%).

Amongst new cases, more than half of the patients (59.8%) reported vitD deficiency. In addition, 333 (22.2%) patients had insufficient level of vitD. VitD deficiency was reported in only 15.2% patients. The highest number of patients with newly diagnosed vitD deficiency was reported in patient with T2DM (63.0%), followed by those with combined condition of T2DM and HT (60.2%) and HT only (56.3%).

Amongst old cases, only 16 (1.1%) patients reported vitD deficiency. Moreover, 10 (0.7%) and 16 (1.1%) patients had insufficent and suficient level of vitD, respectively. Amongst cohorts, vitD deficiency was highest in those who had combined condition of T2DM and HT (1.4%), followed by T2DM only (1.0%) patients.

Table 1: Baseline and Demographics Characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T2DM (N=504)</th>
<th>HT (N=501)</th>
<th>T2DM and HT (N=503)</th>
<th>Overall (N=1508)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years), Mean (SD)</td>
<td>50.7 (12.18)</td>
<td>51.6 (13.58)</td>
<td>56.4 (10.82)</td>
<td>52.9 (12.49)</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>240 (47.62)</td>
<td>283 (56.49)</td>
<td>269 (53.48)</td>
<td>792 (52.52)</td>
</tr>
<tr>
<td>Men</td>
<td>264 (52.38)</td>
<td>218 (43.51)</td>
<td>234 (46.52)</td>
<td>716 (47.48)</td>
</tr>
<tr>
<td>Education, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graduate or Post Graduate</td>
<td>233 (46.23)</td>
<td>232 (46.31)</td>
<td>220 (43.74)</td>
<td>685 (45.42)</td>
</tr>
<tr>
<td>High School Certificate</td>
<td>79 (15.67)</td>
<td>87 (17.37)</td>
<td>75 (14.91)</td>
<td>241 (15.98)</td>
</tr>
<tr>
<td>Intermediate or post high school diploma</td>
<td>119 (23.61)</td>
<td>121 (24.15)</td>
<td>117 (23.26)</td>
<td>357 (23.67)</td>
</tr>
<tr>
<td>Middle School Certificate</td>
<td>43 (8.53)</td>
<td>31 (6.19)</td>
<td>51 (10.14)</td>
<td>125 (8.29)</td>
</tr>
<tr>
<td>Primary School Certificate</td>
<td>30 (5.95)</td>
<td>30 (5.99)</td>
<td>40 (7.95)</td>
<td>100 (6.63)</td>
</tr>
<tr>
<td>BMI (Kg/m2), Mean (SD)</td>
<td>26.3 (4.24)</td>
<td>27.1 (4.60)</td>
<td>27.4 (4.54)</td>
<td>26.9 (4.49)</td>
</tr>
<tr>
<td>BMI Categorization, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>97 (19.25)</td>
<td>73 (14.57)</td>
<td>70 (13.92)</td>
<td>240 (15.92)</td>
</tr>
<tr>
<td>Obese</td>
<td>303 (60.12)</td>
<td>333 (66.47)</td>
<td>349 (69.38)</td>
<td>985 (65.32)</td>
</tr>
<tr>
<td>Overweight</td>
<td>88 (17.46)</td>
<td>86 (17.17)</td>
<td>78 (15.15)</td>
<td>252 (16.71)</td>
</tr>
<tr>
<td>Underweight</td>
<td>16 (3.17)</td>
<td>9 (1.80)</td>
<td>6 (1.19)</td>
<td>31 (2.06)</td>
</tr>
</tbody>
</table>

T2DM: Type-2 diabetes mellitus; HT: hypertension

Table 2: Low level of vitamin D in Patients with T2DM, HT and Both T2DM and HT (N=1501)

<table>
<thead>
<tr>
<th>Vitamin D, n [%95% CI]*</th>
<th>T2DM (N=500)</th>
<th>HT (N=499)</th>
<th>Combined condition of T2DM and HT (N=502)</th>
<th>Overall (N=1501)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>421 (84.2%)</td>
<td>412 (82.6%)</td>
<td>424 (84.5%)</td>
<td>1257 (83.7%)</td>
</tr>
<tr>
<td>(New + Old)</td>
<td>[80.70:87.29]</td>
<td>[78.95:85.79]</td>
<td>[80.99:87.52]</td>
<td>[81.78:85.58]</td>
</tr>
<tr>
<td>New Cases</td>
<td>411 (82.2%)</td>
<td>406 (81.4%)</td>
<td>414 (82.5%)</td>
<td>1231 (82.0%)</td>
</tr>
<tr>
<td>[78.56:85.45]</td>
<td>[77.66:84.69]</td>
<td>[78.86:85.70]</td>
<td>[82.41:86.20]</td>
<td>[82.41:86.20]</td>
</tr>
<tr>
<td>Old Cases</td>
<td>10 (2.0%)</td>
<td>6 (1.2%)</td>
<td>10 (2.0%)</td>
<td>26 (1.7%)</td>
</tr>
<tr>
<td>[0.96:3.65]</td>
<td>[0.44:2.60]</td>
<td>[0.96:3.63]</td>
<td>[45.64:76.43]</td>
<td></td>
</tr>
</tbody>
</table>

T2DM: Type-2 diabetes mellitus; HT: hypertension * Patients with low level of vitamin D (Serum 25(OH) D =<30 ng/ml) were considered.
Vitamin D and hypothyroidism

In this study, 76.9% of hypothyroid patients had low level of vitD (<30 ng/ml). Significant association was noted between different levels of vitD and hypothyroidism (p< 0.0001) (Table 4).

Vitamin D and glycemic indices

No significant association between different levels of vitD and FPG, PPG, or HbA1c in patients with T2DM and T2DM+HT was noted.

Out of 126 patients with low vitD levels and T2DM, 84.37% patients had abnormal HbA1c levels. In patients with T2DM, with vitD insufficiency or deficiency, higher number of patients had abnormal HbA1c value as compared to normal HbA1c values.

Pharmacological Management for vitamin D deficiency

A total of 1066 out of 1257 (84.8%) patients with low level of vitD were prescribed with vitD supplements. Majority of patients (883 [70.2%]) received 60,000 IU dose of vitD. The preferred route of administration was oral (79.6%), once in a week (76.7%). The mean duration of treatment was 7.6±3.49 weeks.

VitD deficiency (406 [26.9%]) and insufficiency (521 [34.5%]) levels, followed by symptoms of vitD deficiency (157 [10.4%]) were the major factors considered by physicians in prescribing vitD supplements to the patients.

Discussion

Research indicate that vitD deficiency is associated with an increased risk of developing T2DM and HT, thus leading to cardiovascular, and cerebrovascular diseases [21,22]. Majumdar et al recently reported that there is 3.13-fold increased risk of ischemic stroke associated with low vitD levels in patients with HT [21]. This rationale calls for routine evaluation of vitD deficiency in patients with Type-2 Diabetes Mellitus and Hypertension - A Cross-sectional, Observational, Pan-India Study

Table 3: Vitamin D levels (deficient, insufficient and sufficient) in newly and previously diagnosed cases of T2DM, HT and combined condition of T2DM and HT

<table>
<thead>
<tr>
<th>Vitamin D Levels, n(%) [95% CI]</th>
<th>T2DM (N=500)</th>
<th>HT (N=499)</th>
<th>Combined condition of T2DM and HT (N=502)</th>
<th>Overall (N=1501)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficiency</td>
<td>320 (64.0%)</td>
<td>285 (57.1%)</td>
<td>309 (61.6%) [57.14:65.83]</td>
<td>914 (60.9%) [58.37:63.37]</td>
</tr>
<tr>
<td>Insufficiency</td>
<td>101 (20.2%)</td>
<td>127 (25.5%)</td>
<td>115 (22.9%) [19.30:26.84]</td>
<td>343 (22.9%) [20.75:25.06]</td>
</tr>
<tr>
<td>Sufficiency</td>
<td>79 (15.8%)</td>
<td>87 (17.4%)</td>
<td>78 (15.5%) [12.48:19.01]</td>
<td>244 (16.3%) [14.42:18.22]</td>
</tr>
<tr>
<td>New Cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficiency</td>
<td>315 (63.0%)</td>
<td>281 (56.3%)</td>
<td>302 (60.2%) [55.73:64.47]</td>
<td>898 (59.8%) [57.30:62.32]</td>
</tr>
<tr>
<td>Insufficiency</td>
<td>96 (19.2%)</td>
<td>125 (25.1%)</td>
<td>112 (22.3%) [18.74:26.21]</td>
<td>333 (22.2%) [20.11:24.37]</td>
</tr>
<tr>
<td>Sufficiency</td>
<td>73 (14.6%)</td>
<td>81 (16.2%)</td>
<td>74 (14.7%) [11.76:18.15]</td>
<td>228 (15.2%) [13.41:17.11]</td>
</tr>
<tr>
<td>Old Cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficiency</td>
<td>5 (1.0%)</td>
<td>4 (0.8%)</td>
<td>7 (1.4%) [0.56:2.85]</td>
<td>16 (1.1%) [0.61:1.73]</td>
</tr>
<tr>
<td>Insufficiency</td>
<td>5 (1.0%)</td>
<td>2 (0.4%)</td>
<td>3 (0.6%) [0.12:1.74]</td>
<td>10 (0.7%) [0.32:1.22]</td>
</tr>
<tr>
<td>Sufficiency</td>
<td>6 (1.2%)</td>
<td>6 (1.2%)</td>
<td>4 (0.8%) [0.22:2.03]</td>
<td>16 (1.1%) [0.61:1.73]</td>
</tr>
</tbody>
</table>

T2DM: Type-2 diabetes mellitus; HT: hypertension; aSufficiency: Serum 25(OH)D ≥ 30 ng/ml; bInsufficiency: Serum 25(OH)D between 21-29 ng/ml; cDeficiency: Serum 25(OH)D ≤ 20 ng/ml.

Table 4: Association between Different Categories of VitD Levels and Hypothyroidism

<table>
<thead>
<tr>
<th>Vitamin D levels</th>
<th>Number of patients (%)</th>
<th>Hypothyroidism, number of patients (%)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Presence (n=436)</td>
<td>Absence (n=1065)</td>
<td></td>
</tr>
<tr>
<td>Deficiency</td>
<td>913(60.8%)</td>
<td>224 (51.4%)</td>
<td>689 (64.7%)</td>
</tr>
<tr>
<td>Insufficiency</td>
<td>343 (22.9%)</td>
<td>111 (25.5%)</td>
<td>232 (21.8%)</td>
</tr>
<tr>
<td>Sufficiency</td>
<td>244 (16.3%)</td>
<td>101 (23.2%)</td>
<td>143 (13.4%)</td>
</tr>
</tbody>
</table>

T2DM: Type-2 diabetes mellitus; HT: hypertension; * calculated using chi square test.
with T2DM and HT to tailor prevention strategies and early treatment. Further, the growing burden of T2DM and HT and the increasing prevalence of low level of vitD has apprehended the attention of health care practitioners, particularly in countries with co-occurrence of T2DM and HT.

Even though vitD deficiency prevails in epidemic proportions all over the Indian subcontinent with a prevalence range of 70% to 100% in general population, [23] very little information exists about its prevalence and their clinical practice management among patients with T2DM, HT, and those with co-occurrence of T2DM and HT in India. Our study is the first of its kind attempt to provide critical insights into the disease burden of vitD deficiency, its association with hypothyroidism and their management strategy in the cohort of T2DM, HT, and both T2DM+HT in India.

In this cross-sectional study, the overall prevalence of patients with low vitD levels (deficiency and insufficiency) was 1257 (83.7%). Out of this, 1231 (82%) were newly diagnosed cases. This high prevalence of vitD deficiency in newly diagnosed cases indicates that vitD deficiency may have been missed in a large proportion of patients with T2DM, HT, or both T2DM and HT. The proportion of patients with low vitD in patients with T2DM, HT and both T2DM + HT was found to be 84.2%, 82.6% and 84.5%, respectively. Our results were in agreement to the prevalence reported in other studies from India. A recent study among T2DM patients reported 71.4% patients with vitD deficiency and 15% with insufficiency in South India region [22]. Among patients with low levels of vitD, deficiency was observed in nearly two-third (60.9%) of patients, while insufficiency was noted in remaining (22.9%) patients. Sheth et al observed vitD deficiency in 91.4% of cases of T2DM and 93% in the control group. However, they could not establish any association between vitD deficiency and glycated haemoglobin [24]. Kumar et al in a study from Pondicherry observed vitD deficiency in 32% of cases with T2DM and 25% of controls. Further, low VitD levels were observed in 66.5% of the study population (including cases and controls) [25]. In another study by Kumar H et al, the prevalence vitD deficiency among patients with T2DM from Rajasthan was found to be 91.1% [26]. On the other hand, the prevalence of vitD deficiency in hypertensive patients from North India was found to be 80.4% [27].

This study reported a significant association between levels of vitD and hypothyroidism (p<0.0001). In agreement with this observation, Talaei et al. (2017) also reported that the lack of vitD contributed to the possibility of low thyroid hormones [28]. Additionally, a recent cross-sectional study correlated the deficiency of vitD with increased levels of TSH in hypothyroidism patients [29]. This could be attributed to the variation in the vitD receptor gene, which are thought to mediate susceptibility to various endocrinial autoimmune diseases. Further, it has also been proposed that the decrease in the level of vitD in hyperthyroid patients could result in increased level of calcium, resulting in a negative impact on the secretion of parathyroid hormone and vitD synthesis [30].

In this study, a non-significant inverse correlation was observed between different levels of vitD and HbA1c T2DM and T2DM+HT (T2DM: \( r = -0.10, p = 0.21 \); T2DM+HT: \( r = -0.10, p = 0.23 \)). Similar results were noted by Calvo-Romero and Ramiro-Lozano (2015), where inverse correlation between serum levels of 25(OH)D and glycosylated hemoglobin (\( r = -0.74, P = 0.01 \)) were reported [33]. Another study demonstrated similar negative correlation association between vitD level and HbA1c (P = 0.035) in patients with diabetes [34]. Brijesh et al. observed 25 (OH)D levels of 23.63±3.71 ng/ml in patients with T2DM with HbA1c levels less than 7 g% and 25OH vitD levels of 19.41±4.76 ng/ml in patients with T2DM with HBA1c levels more than 7 g% [35]. This implies that supplementation of vitD could be effective in improving glycemic control in patients with T2DM with deficient or insufficient levels of vitD.

A total of 84.8% patients with low level of vitD were prescribed with vitD supplements. Most of the patients in our study were prescribed a dose of 60000 IU (70.2%) via oral route (79.6%) once a week (76.7%). Physicians often prescribe 1500mg (60 000 IU) cholecalciferol per week for 8 weeks for vitD deficiency in India [36]. Nevertheless, data on maintenance therapy of vitD supplements to prevent recurrence of vitD deficiency, after treatment of acute deficiency, is lacking. Pietras SM et al showed that 50,000 IU of ergocalciferol weekly for 8 weeks is effective in treating vitD deficiency, and continued treatment with 50,000 IU of ergocalciferol every alternate week for up to 6 years prevents recurrent vitD deficiency in most patients [37]. However, commercially, ergocalciferol (50,000IU) preparation is not available in India. Hence, 60,000 IU cholecalciferol every alternate week for up to 6 years is an effective maintenance therapy, after treatment of acute deficiency, to prevent vitD deficiency in Indian patients.
Our study has strengths and limitations. The strengths being that this is the first study highlighting the burden of vitD deficiency in patients with co-occurrence of T2DM and HT. The results can be generalized as it is a Pan-India study covering a large number of patients across the different geographical region and provided critical insights into the disease burden of vitD deficiency in patients with T2DM and/or HT in India. One of the limitation of this study is that no control group was included to see the viability of results in studied population. Nevertheless, this is the first of its kind nationwide data on the prevalence of vitD deficiency and its association with hypothyroidism in patients with T2DM and/or HT. However, a longitudinal study is warranted to ascertain the long-term association between vitD deficiency and T2DM and/or HT and to assess its impact on disease progression. Additionally, randomized controlled trials are required to provide an insight into the efficacy and safety of vitD as a therapeutic tool for vitD deficiency in such patients.

Conclusion

A high prevalence of vitD deficiency was found in patients with T2DM, HT and T2DM+HT in India. Prevalence of vitD deficiency was higher amongst newly diagnosed cases compared to already diagnosed cases (in all three cohorts [T2DM/HT/T2DM and HT]). This could indicate that vitD deficiency may have been missed in a large proportion of patients with T2DM, HT, or both T2DM and HT. The low level of vitD and its association with hypothyroidism was also evident from our findings. This study thus underscores the magnitude of overlap between vitD deficiency and T2DM or HT, and the need for routine vitD screening for early diagnosis and effective management.

Conflict of Interest: Dr. Talwalkar, Dr. Deshmukh, Dr. Deepak and Dr. Agrawal received research funding from Abbott India. Ltd.

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References


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Comparative Evaluation of Metformin and Letrozole in a Rat Model of Polycystic Ovary Syndrome

Chakraborty Pratip¹, Chatterjee Sujan², Ipsita Chaterjee³, Patra Debajyoti⁴, Chakravarty Baidyanath⁵, Kabir Syed N⁶

How to cite this article:

Abstract

Background: A number of rat models have been proposed to investigate the heterogeneity of polycystic ovary syndrome (PCOS), hyperandrogenized routine being the most popular one. Although, effects of androgen excess have been widely investigated, the ameliorating potential of the ovulogens on different aspects of the treatment on a single model is yet to be explored. Aims: This particular experimental study ends to investigate the effects of metformin and letrozole on experimentally induced rodent model of PCOS. Methods and materials: After neonatal androgenisation (1.5 mg/animal), rats were given metformin (200 mg/kg body weight for 4 weeks) or letrozole (0.5 mg /kg/day for 15 days) from ~53 day of age. Ovarian histology, response to exogenous gonadotropins, hormonal profile, glucose tolerance, and dyslipidaemic features with RT-PCR of specific steroidogenic and hyperinsulinemic genes were carried out to compare and evaluate the therapeutic response of the drugs. Results and conclusion: Androgenized rats had polycystic ovaries characterized by increased theca interna and fewer or no functional corpora lutea. Metformin improved ovarian responses and was superior in attenuating testosterone status, hyperinsulinemia, dyslipidaemia along with a significant improvement in expression of the genes assessed. In conclusion, metformin reverted the hyperinsulinemia-associated hyperandrogenemia to reinstate ovulatory function with parallel improvements in the metabolic corners of the syndrome; however, letrozole appears to have no beneficial effect with reference to restoration of ovulation or amelioration of polycystic state of the ovary.

Keywords: polycystic ovary syndrome; ovulation induction; hyperinsulinemia; hyperandrogenemia; animal model.

Introduction

Polycystic ovary syndrome (PCOS) is a major form of dysovulatory infertility that affects 5-10% of women of reproductive age (Sattar et al., 2009). The syndrome is a heterogeneous entity comprising broad spectra of ovarian disorders and metabolic syndrome (MS) including anovulation, hyperandrogenism with frequent association of insulin resistance (IR), obesity and dyslipidaemia (Escobar-Morreale et al., 2005) to name a few. Hyperandrogenism is believed to be at the heart of the syndrome since excess androgens from multiple small follicles results anovulation and IR (Homburg et al., 2009). Therefore, medical therapy is often aimed at lowering the hyperandrogenic and infertile status.

PCOS women, in general have difficulty becoming pregnant due to anovulation. Induction of ovulation is the first-line of treatment for these women and is aimed at the introduction of an endocrine milieu that promotes growth and ovulation of a single dominant follicle with consequent singleton pregnancy.
Clomiphene citrate (CC) has been the gold standard treatment option for induction of ovulation in women with PCOS for many decades (Neveu et al., 2007). Discrepancy between ovulation and pregnancy rates with CC has been attributed to its anti-estrogenic action and estrogen receptor depletion at the endometrium level. In this context aromatase inhibitors (AI) have emerged as an alternative treatment to clomiphene. Letrozole, the most prevalently used anti-aromatase for this indication, has been shown to be effective. The drug is non-steroidal, highly potent, well tolerated competitive inhibitor of aromatase enzyme system (Casper et al., 2006).

As hyperinsulinemia plays a significant role in anovulation in PCOS, clinical improvements can be anticipated following the reduction of serum insulin concentrations (Tang et al., 2012). Metformin acts by decreasing gluconeogenesis as well as increasing peripheral utilization of glucose in the presence of endogeneous insulin. A number of studies (Genazzani et al., 2007; Elter et al., 2005; Pasquali et al., 2006) have shown significant improvements in insulin sensitivity and hyperinsulinemia in non-obese PCOS women after metformin administration. Furthermore, these changes were associated with a reduction in serum androgen and a concomitant increase in serum sex-hormone-binding globulin concentrations. Recent report also document metformin usage for ovulation induction in PCOS women promote ovulation and pregnancy rate which is comparable to that of clomiphene (Xiao et al., 2012).

A number of rat models have been proposed to investigate the heterogeneity of the syndrome, hyper-androgenized routine being the most popular one (Amalﬁ et al., 2012). To our knowledge, no studies have been performed to compare the effects of metformin and letrozole on testosterone-induced PCOS in an animal model. Additionally, there has been no research to determine the effect of letrozole on the metabolic corner of PCOS in a rat model. Thus, the aim of the present study was to evaluate the potential of metformin and letrozole in improving both endocrine as well as metabolic milieu in androgen induced rodent PCOS model.

**Materials and Methods**

**Animals: Maintenance and Care**

Adult healthy female Sprague Dawley rats (*Rattus norvegicus*, 180-220g) were housed in controlled conditions of room temperature (23±2°C), humidity (50±5%), and a 12 h-12 h light-dark cycle. The animals were kept in sanitized polypropylene cages and fed with standard rat pellet diet and drinking water *ad libitum*. All experiments were performed as per the national guidelines formulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Culture, India with approval from the Animal Ethics Committee of Institute of Reproductive Medicine.

**Chemicals**

Unless otherwise stated, chemicals used for the preparation of reagents were of analytic grade and purchased from Sigma Aldrich (Sigma Aldrich, St. Louis, M.O., USA). HEPES, Dulbecco’s Modified Eagle’s Medium and antibiotics were purchased from Invitrogen (Life Technologies, Grand Island, NY, USA). Letrozole was purchased from (Femara, Novartis, US) Radioimmunoassay (RIA) kits for total testosterone (DSL-4000 Active® Testosterone Coated-Tube RIA Kit) and estradiol-17β (DSL-4400 17β-Estradiol RIA Kit) were obtained from Diagnostic Systems Laboratories (DSL, Webster, Texas, USA). Follicle stimulating hormone (RK-550) and luteinizing hormone (RK-552) kits were procured from IZOTOP (Institute of Isotopes Ltd, Budapest, Hungary), while RIA kit for rat insulin was procured from BIOTRAK (AP Biotech, Amersham, UK).

**Induction of PCO by neonatal androgenisation and subsequent treatment design**

A single subcutaneous (SC) injection of testosterone propionate (TP) (Nutritional Biochemical Corporation, Cleveland, USA) dissolved in olive oil was administered at a dose of 1.5 mg/animal on day 5 to induce PCO Ota et al, 1986. Control animals were injected with olive oil alone. At 53±1 day of age, the TP-treated rats were divided into 3 groups each comprising 10 rats. Group 1 (PCO) was given no medication. The other two groups were orally treated with 200 mg/kg body weight of oral metformin for 4 weeks or 0.5 mg/kg/day of oral letrozole for 15 days. A separate group (Group 4) consisting of 10 rats was maintained as control. The medications were given via orogastric tubes.

**Evaluation of growth rate**

The litters from different groups were weighed every seventh day since birth, continuing throughout the course of experiments.
Histoarchitecture of the ovary

After completion of the treatment regimen a section of animals from each group were killed under ether anaesthesia. The ovaries were dissected out and fixed by immersion in 3.7% buffered formaldehyde in 0.1M phosphate buffer, pH 7.2. After fixation, the tissues were processed, embedded in paraffin and serially sectioned at 5µm with a Leica RM2135 rotator microtome. Sections from the ovaries were used for routine haematoxylin-eosin staining and photographed under a Biomed light microscope enabled with a Leica RM2235 digital camera.

Ovarian response to exogenous gonadotropins

At 10-12 weeks of age, rats (n = 7) per group were fasted overnight and experimental groups were induced to superovulate by s.c. injection of 40 IU PMSG at '0' h and 25 IU hCG at ‘52’ h after the completion of respective treatments. Oviducts were flushed with normal saline after '20' h when the presence of oocytes and their numbers were recorded. Follicular response was assessed both in respects of the number of rats (%) responded and the number of oocytes retrieved.

Hormonal analyses

At ~ 12 weeks of age blood samples were collected by cardiac puncture under ether terminal anaesthesia in vacutainer with or without anticoagulant. Hormones were measured by RIA using the protocol described in the respective instruction manuals. The intra- and inter-assay coefficients of variation were within the acceptable limits (<10%). Hormone levels were expressed as ng/ml or pg/ml, as applicable.

Oral Glucose Tolerance Test (OGTT)

At 10-12 weeks of age, insulin sensitivity was examined by performing OGTT. OGTT was performed according to the standard protocol Du Vigneaud et al, 1925. Briefly, the rats (n = 7 from each group) were fasted overnight and next day bled at ‘0’ hr to estimate the fasting glucose level followed by an oral glucose challenge (2 g/kg body weight). Animals from the either group had ~0.02 mL of blood drawn (by tail nick) at 30, 60, 90, 120 and 180 minutes (min) after glucose administration and blood glucose levels were measured using a commercial glucometer (Aku-check, sensor comfort, Roche Diagnostics, Germany).

Biochemical Analysis of Lipid Profile

At 10-12 weeks of age blood was obtained by cardiac puncture after an overnight fasting to assess the lipid profile. Serum total cholesterol, triglyceride and lipoprotein levels were measured by automatic enzymatic methods in all groups. HDL was determined after precipitation of the lipoproteins by dextran sulphate Ruel, et al. 2003. The serum levels of total cholesterol, HDL and triglyceride (TG) levels were measured using reagents purchased from Randox Laboratories Ltd, London, UK by Daytonia. Lipoprotein levels were expressed in mg/dl. LDL cholesterol was calculated using the Friedewald formula: LDL-C = cholesterol-HDL-C-(TG/5). Serum samples were stored at -20°C until assayed.

Semiquantitative Polymerase Chain Reaction

Total RNA was extracted from five ovaries for each group using Trizol reagent (Invitrogen, California, USA) and was reverse transcribed into complementary DNA (C_DNA) in a total volume of 20µL reaction mixture containing 1µl oligo (dT)_{12,18} (500 ng/µl) (Applied Biosystems). Reverse transcriptase was performed with 200U/µl superscript II reverse transcriptase (Invitrogen) at 42°C for 60 min. C_DNA amplification was carried out in an automated thermal cycler (iCycler; Bio-Rad) using the appropriate conditions for StAR (forward 5’-ggcatcttgaacacagga-3’; reverse 5’-tctcttgacatgatggtgc-3’; Tm 56°C), insulin-like growth factor-1 (IGF-1)(forward 5’-tcgctcttactedtctctctcttc-3’; reverse 5’-acactccagctctcaga-3’; Tm 55°C) and Cyp19 (forward 5’-attctttgagggattt-3’; reverse 5’-acagctcggtgtttgta-3’; Tm 55°C) primers. β-actin was used as positive control. An aliquot of each C_DNA sample was amplified by PCR with β-actin gene-specific primers (forward 5’-cattgctggggacagttggttta-3’; reverse 5’-taacctgacacatcctcggg-3’; Tm 55°C). Upon completion of the PCR reaction, products (10µl) were subjected to electrophoresis on 1.5% agarose gel and in Tris acetate- EDTA buffer and stained with ethidium bromide (0.5µg/ml). The relative amount of the transcript was calculated by dividing the intensity of the band by the intensity of β-actin.

Statistical Analysis

The data were analyzed as mean±standard error of the mean (SEM), where ‘n’ refers to the number of animals or determinations. The results were analyzed by paired two-tailed t-test using the GraphPad Prism 3.0 software (Graph Pad Software, Inc, San Diego, CA). Densitometric quantification of
signals by RT-PCR was done by Image-J software. All values were normalized with internal control, i.e. β-actin, and represented in the form of bar diagram. p<0.05 was considered significantly different.

Results

Reduction of body weight after metformin regimen

Body weights (BW) of the litters at birth and subsequent growth rates have been presented in Figure 1. At birth the BW of the pups were statistically indifferent between the groups, although during the course of time, PCO pups showed relatively higher rate of weight gain at different points of time from 7th week onwards (Fig. 1A). The metformin treated group, by contrast, showed reduced growth rate (p<0.04) as compared to that of the TP-intervened study group in the later stages of treatment.

Ovarian response to gonadotropins

Ovarian response to gonadotropins was evaluated with respect to the number of animals (%) exhibiting the presence of ovulated eggs in the oviduct, as well as the number of ovulated eggs per stimulated rats. All control rats (100%) responded to stimulation at a rate of 34.42±3.52 ovulated eggs per rat. By contrast, PCO group of rats responded very poorly (p < 0.0002) to the stimulation. PCO group, 20% of the rats did not respond to stimulation, while the rest responded with 9.33±1.85 of ovulated eggs per rat (Fig. 1B). All rats responded well with metformin treatment significantly improved (p<0.002) the ovarian response (26.2±3.05) in comparison to 60% of the rats that received letrozole treatment displaying marginal beneficial effect (p < 0.05) when compared to the androgenised set (16.6±2.51).

Glucose tolerance

Figure 1C represents the outcome of OGGT. PCO group had 92.46±2.31 mg/dL of basal blood glucose, which was significantly greater (p<0.03) than that of control (83±1.86). As compared with control, the glucose levels at all-time points were higher in the androgenised group. However, in both control and PCO groups plasma glucose levels reached their

Fig. 1: Comparative evaluation of growth rate, ovarian response to gonadotropins and glucose level in different groups

A. Postnatal growth rates in the control, TP-treated and drug treatment groups. As compared to controls the rate of growth in the androgenised rats are comparatively higher (p < 0.002; p<0.0006; p<0.03) during the 7-10 postnatal weeks. However, metformin management showed reduced growth rate (p<0.04), when compared to that of control. Each point represents the mean±SEM values of 8-10 different animals.*p<0.03: PCO vs Control; **p<0.002: PCO vs Control; ***p<0.0006: PCO vs Control, #p<0.04 PCO vs Metformin.

B. Ovarian response to standard PMSG/hCG stimulation is presented as number of ovulated oocytes (mean ±SEM)/rat. The androgenised group shows significantly (p<0.0002) lower number of ovulated oocytes/ rat compared to control; however, rats treated with metformin (p<0.002) or letrozole (p<0.05) significantly improved the ovarian response. Each bar represents the mean±SEM of five individuals observations of the respective group.***p<0.0002: PCO vs control; **p<0.002: PCO vs Metformin; **p<0.005: PCO vs Letrozole.

C. Plasma glucose levels after glucose challenge at 2 g/kg body weight after different treatment regimens with comparison to control set. The androgenised group exhibit poor glucose tolerance. Metformin could only recover the poor glucose tolerance of the androgenised set. Each datum point represents the mean±SEM of 5-7 observations in the respective group. */** mark the level of significance, while the superscripts designate the groups compared. *control vs TP; **TPs vs metformin; *p<0.03; **p<0.006; ***p<0.0002.
peak at 60 min and came down at a comparatively lower rate to maintain significantly higher (p<0.03) glucose levels over their basal values until 180 min. However, significant improvement were observed in fasting blood glucose level on completion of metformin treatment (p<0.03) which continued during the course of glucose tolerance over other groups. The metformin-treated group showed the peak sugar level at 60 min that decreased to the basal level by 120 min, while letrozole-treated group maintained high levels at least until 180 min.

**Ovarian architecture**

Light microscopic analysis of the control ovary showed follicles and corpus lutea in different stages of development and regression (Fig. 2A). Polyhedral theca and cuboidal granulosa cells were observed in control. By contrast, TP rat ovaries showed increased number of follicular cysts characterized by large fluid filled cavity with disorganized granulosa cell layers and thickened theca interna with the presence of primary and early secondary follicles (Fig. 2B). Treatment with metformin (Fig. 2C) made considerable recovery of ovarian architecture as marked by the presence of corpus luteum as a sign of ovulation. Letrozole (Fig. 2D) led to arrest of follicle growth with comparable number of pre-antral follicles and cysts to that of the hyper androgenised set.

**Serum hormone analysis and correction of dyslipidemia**

The serum gonadotropin profile of the PCO group demonstrated a significant decrease in FSH concentration (PCO vs. control p<0.04) with an increase (p<0.0001) in testosterone level. However, LH and estradiol level remained statistically comparable to controls with an increase (p<0.04) in insulin (Table 1). Treatment with metformin significantly (p<0.03) reduced the serum testosterone levels of the PCOS rats; however, letrozole had no effect on the same. A significant decrease in insulin (p<0.03) and estradiol (p <0.01) was observed after treatment with metformin and letrozole, respectively, as compared to the androgenised set.

Table 2 summarizes the lipid profiles in the control and study groups. PCO rats had higher levels of triglyceride (p<0.003) compared with controls. Total cholesterol was comparable in PCO group. As compared with the control, PCO set exhibited comparatively lower level of HDL (p<0.02) and higher level of LDL, however the difference in the latter was not significant enough. Letrozole treatment had no influence on the lipid profile of the TP-treated rats. Metformin treatment, however, changed the lipid profile significantly by reduction in triglyceride (p<0.02), with an increment in HDL cholesterol (p<0.02).

**Expression of StAR, IGF-1 and Cyp19**

The PCO group demonstrated increased ovarian expression of StAR, Cyp19 (p<0.001) and IGF-1 (p<0.001)(Fig. 3) when compared to control. However, a significant decrease in StAR expression (p<0.01) with a simultaneous diminution (p<0.02) in Cyp 19 profile was observed in the metformin-treated group. Letrozole treatment...
also documented a significant decrease in Cyp19 (p <0.001) expression. An up-regulation of IGF-1 expression was noted in the hyperandrogenized group, which got significantly decreased after treatment with metformin (p<0.001) (Fig 3). Nevertheless, no change in IGF-1 was observed after letrozole regimen.

Discussion

Medical treatments for PCOS usually revolve around the correction of hyperandrogenemia and improvement of ovulation rates. The present results showed comparative superiority of metformin as an ovulogen over letrozole in PCOS.

The PCO set of animals developed ovarian-endocrine abnormalities with elevated serum testosterone (T) concentrations consistent with previous findings. (Franks et al., 1995; Mannerås et al., 2007). Our findings suggest supremacy of metformin over letrozole in reducing hyperandrogenicity with improving the metabolic set-backs of androgenised set.

Sex steroids appear to be important in regulating adiposity cueing the result of TP treatment to a significant increase in body weight. Moreover, administration of testosterone has got its direct correlation with IR attributed to effects on glucose transport and/or glucose intolerance (Rincon et al., 1996). Metformin reduces the body weight via a direct inhibition in StAR or IGF-1 as well as brought down glucose to basal level by 120 min indicating improved glucose tolerance as perceived in corresponding human studies (Lehtovirta et al., 2001) however with no improvement after letrozole regimen. This may render a strong, more direct effect of metformin on glucose tolerance so as to reduce hyperandrogenicity with a concomitant improvement in response to exogenous gonadotropins.

Our androgen-induced rodent model displayed an elevated LH/FSH ratio which was achieved through lowering of FSH with unaltered serum LH levels and a significant increase in insulin level. A well-known positive correlation exists between insulin and androgen in women with PCOS (Wang et al., 2001). Metformin via its insulin sensitizing effect and by direct inhibition of androgens improves insulin sensitivity (Mansfield et al., 2003). The inhibition of aromatase by metformin might be via an extracellular signal regulated kinase pathways recently proposed by (Rice et al., 2009). Approximately 70% of PCOS patients have dyslipidaemia characterized by increased total and low density lipoprotein-cholesterol (LDL-C) and triglyceride (TG) (King et al., 2007; Legro et al., 2001) observed that exposure to excess T may alter serum TG and LDL levels in a manner similar to that of lean PCOS women irrespective of obesity (Legro et al., 2001). Augmented TG level was reversed to normal after metformin treatment. This might have led to withdrawal of inhibitory effects of IR on high-density lipoprotein-cholesterol synthesis and therefore reverted back the atherogenic lipid profile to normal one.

Letrozole had been used for the development of PCOS and has proven hyperandrogenic effects in many experimental rat models (Mannerås et al., 2007); hence the usage of the drug for the amelioration of the metabolic and ovulatory disturbances in experimentally induced hyperandrogenic milieu may be a conflicting methodology. However,

Fig. 3: Ovarian expression of gens following treatment regimen/s
A. RT-PCR analysis of ovarian expression of StAR, Cyp 19 and IGF-1 of hyperhomocysteinemic rats following different treatment regimens. β-Actin is showed as a loading control. B. Densitometric analyses of the RT-PCR by Image J software.
aromatase inhibitors are widely recommended for inducing ovulation in anovulatory women with PCOS after CC (Nader et al., 2007). Incidentally an increased rate of bone and cardiac anomalies in fetuses born to women after letrozole treatment was reported. Nevertheless, extensive examination of this issue has failed to substantiate these findings (Pritts et al., 2010). On the other hand, the safety of metformin therapy sounds theoretically appealing due to the lack of serious side effects like raised androgen levels observed due to hyperoestrogenism in cases with letrozole. Recently, a metaanalysis of randomized clinical trials also revealed that metformin on its own or with CC was significantly superior to placebo or CC alone for ovulation induction (Xiao et al., 2012).

Metformin is well known to reduce hyperinsulinaemia and hyperandrogenaemia facilitating normal menses and pregnancy (Moghetti et al., 2000). However, the molecular mechanism of action of metformin remains uncertain. The action of metformin in muscle cells is via its easing of glucose transport and activation of tyrosine kinase activity mediated by IGF-1. Androgens increase the expression of IGF-1, and IGF-1 receptor in primate follicles and oocytes (Vendola et al., 1999). Hence, we evaluated the expression of StAR and IGF-1. Hyperinsulinaemia, observed in our model could inhibit IGF-1-binding protein production by the liver. As a result, unbound IGF-1 in conjunction with LH could stimulate ovarian thecal cell androgen production (Stadmayer et al., 2002). In the adult ovary, expression of P450scc and StAR genes under the regulation of pituitary gonadotropins, via the hypothalamic-pituitary-gonadal axis feedback system (Chen et al., 2010) Steroidogenesis in the ovarian theca cell is primarily regulated by LH, which upon binding to the LH receptor promotes increased steroid production; gonadotropins activated cAMP-dependent PKA signalling and up-regulated multiple key steps in steroidogenesis, including StAR gene expression (Manna et al., 2009). LH and insulin or insulin-like growth factor (IGF-1) act in synergy in stimulating StAR messenger RNA accumulation (Sekar et al., 2000). Thecal over-expression of StAR (Jakimiuk et al., 2001) improved androgen productivity. Metformin directly inhibits theca production of androgens (Rice et al., 2009) through reduction of IGF-1 and StAR as reflected in our results. Hence, the attenuating effect of metformin on StAR expression may be a direct one (reducing androgens) or a change in the insulin status. However, letrozole do not have any impact on either of the expression profile.

In humans, administration of CC is done during the early follicular phase. Since rats have a 4-5 day cycle length, phase-dependent precise administration of CC is practically difficult, and hence was not attempted.

In summary, our data convincingly demonstrate that regulatory proteins and growth factor mRNAs that are over-expressed and causally associated with development of polycystic ovaries are mostly down regulated following treatment with metformin followed by the reversal of the metabolic arena of the enigma. The use of real time PCR analysis, instead of RT-PCR, however would have provided the array of molecular changes more precisely.

There is little agreement on the ideal treatment of PCOS, which is largely symptom-based. IR plays a vital pathophysiological role in PCOS patients as manifested by causal relationship between IR and the reproductive and metabolic changes of PCOS. Our findings, although limited to androgen-induced PCOS model, demonstrate that letrozole improved the ovarian picture by reducing Cyp19 levels while metformin proves itself as a superior mode of treatment to recover the metabolic disturbances often observed in PCOS patients. In addition, metformin improves the ovarian response to gonadotropins. Based on these results metformin usagemay be useful in infertile patients with PCOS not only for the hyperinsulinemic-PCO subgroup but also for the purpose of induction of ovulation.

**Competing interests:** The author(s) declare that they have no competing interests

**Author’s contributions:** Conceived and designed the experiments: PC, SC. Performed the experiments: PC, IC, SC, DP. Analyzed the data: SNK, BNC. Contributed Reagents/materials/analysis tools: BNC, SNK, PC. Wrote the paper: PC, BNC.

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**IJDAE / Volume 1, Number 1 / January - June 2019**
Obesity and Its Impact on Male and Female Fertility

Joseph RD Fernandes¹, Arnab Banerjee²

Abstract

Obesity constitutes a serious health concern as its increasing rapidly worldwide. What is more alarming is that obesity is increasingly now being observed among all age groups in India. Obesity is gateway to many health issues including impairing fertility of both males and females. It has been observed in obese male and female individuals have altered adipokine and other hormonal profile, with poor quality of sperm and oocyte and varied epigenetic modifications of the germ cells. The review focuses on various aspects of how obesity reduces fertility in both the genders. The review also discusses some of the obesity management procedures which thereby also might help in controlling infertility among obese individuals, particularly, how brown adipose tissue (BAT) might be a focus for new therapeutics for obesity by increasing energy expenditure in obese individuals.

Keywords: Obesity; Fertility.

Introduction

Undernourishment a condition that prevailed in the past due to poverty is rapidly replaced by obesity mostly influenced by industrialization, urbanisation and affluence in India. According to the World Health Organization (WHO) statistics, globally one in six adult people suffer from obesity and nearly 2.8 million people die each year due to either overweight or obesity. A recent study showed that the rate of obesity is constantly increasing redundantly and prevalence of overweight (BMI ≥ 25-29.9 kg/m²) and obesity (BMI ≥ 30 kg/m²) is higher among females than males (Indulekha et al., 2015). If persists, obesity can be the path to many associated metabolic risk factors or metabolic syndromes (MetS) such as non-insulin dependent diabetes mellitus (NIDDM), cardiovascular diseases (CVDs) insulin resistance (Reaven et al., 2004).

Indeed, the etiology of obesity is highly complex which includes genetic, physiologic, environmental, psychological, social which interact in varying degrees to promote the development of obesity (Aronne et al., 2009). In accordance to the International Diabetes Federation in 2006, for a person to be diagnosed with metabolic syndrome, the following criteria have been defined: central obesity measured by waist circumference plus two additional factors such as reduced high density lipoprotein (HDL) cholesterol (<40-50 mg/dl) level, raised triglycerides (>150 mg/dl) level, increased blood pressure (>130 mm Hg systolic or >85 mmHg diastolic) or raised level of fasting plasma glucose (>100 mg/dl) (Eddy et al., 2008).

Undoubtedly, the end results are that humans have a well-defined biological time interval, restricting fertility and reproduction shorter than the life expectancy. Thus, the price of obesity is represented by a long list of comorbidities along with various social, psychological and demographic problems. This exposes both men and women to a greater risk and impact of negative biological and environmental factors. Hence there is a need for urgent novel methods for new weight-loss treatments.

Effects of Obesity on Males

Studies have shown that males suffering from obesity have decreased plasma concentration of

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testosterone, on account of enhanced conversion to estrogen (Kley et al., 1980). The decrease in testosterone is also due to the decrease in sex hormone-binding globulin, SHBG, which may appear be increased due to insulin resistance. Obese men also exhibit an increase in estradiol, estrone, defective estrogen receptors. Also, the excess accumulation of fatty tissue enveloping the scrotum, suprapubic and thigh regions causes hyperthermia or higher scrotal temperatures leads to oxidative stress to the testicles and further has detrimental effects on spermatogenesis (Setchell, 2009). Such undesired scrotal heating on spermatogenesis and fertility are equally evident in non-human primates. Another interesting study on male aging found that overweight men were more prone for erectile dysfunction regardless if they lost weight during the follow-up (Derby et al., 2000).

Overweight and obese men were found to have a 21.6% (95% CI 4%–39.4%) reduction in their sperm concentration compared with normal-weight men. Using flow cytometry, the chromatin integrity in sperm was evaluated and an increase DNA fragmentation is correlated with infertility (Kort et al., 2006). Interestingly a study showed male mice fed with high fat diet displayed altered acetylation status in late round spermatids. Disruptions to the sperm histone acetylation lead to increased DNA damage in mature sperm and potentially correspond to poor sperm parameters that are observed in obese males (Jenkins et al., 2012).

Adipose tissue in addition to be an important organ maintaining balance in energy homeostasis, it also secretes a huge number of cytokines, known as adipokines, such as leptin, adiponectin, resistin, visfatin, etc. (Rosen and Spiegelman, 2006). In obese men, leptin level increases which has an inhibitory effect on the testosterone production. Studies in rodents showed that leptin concentrations at par with that of obese men directly inhibited the conversion of 17OH-progesterone to testosterone. The presence of leptin has been demonstrated in human male spermatocytes in the testes, increased levels of leptin might disrupt spermatogenesis (Ishikawa et al., 2007).

**Effects of Obesity on Females**

Females suffering from obesity contribute relatively a long list of metabolic derangements and further consorted with severe reproductive consequences. Evidence suggests excess of body fat is concorded with increased number of polycystic ovarian syndromes (PCOS), miscarriages, macrosomic fetus, pre-eclampsia, infertility and infertility treatment failure, amenorrhea, multiple complications in pregnancy, gestational diabetes and multiple consequences. Studies have showed that onset of obesity and of menstrual irregularities and oligo-anovulation are significantly correlated between adolescent and young women (Pelusi et al., 2003). The fat distribution in the abdomen may have a specific impact on ovulation and fertility.

Obesity plays a distinct pathophysiological role in the development of hyperandrogenism in women with PCOS and these individuals are characterized by blunted responsiveness to pharmacological treatments to induce ovulation, recurrent miscarriages, reduced incidence of pregnancy and frequent early pregnancy loss. Women with PCOS appear to have a greater prevalence of obesity than expected in the general population. An estimated prevalence rate of more than 30% of cases and, in some series, a percentage as high as 75% exists. Several factors are associated with the complex network relating obesity and PCOS. These factors include insulin, the insulin growth- factor system, the opioid system, estrogens and several cytokines, particularly leptin (Sam 2007).

Obesity drastically impacts the quality of oocyte, which implies lower fertilization rates (Shah et al., 2011). A decline in incidence of embryo transfer and decreased number of transferred embryos have been observed in linear association with increasing BMI. Obesity is also associated with a higher risk of obstetric causes of maternal death as well as anaesthesia-related deaths (Ramsay et al., 2006). Pregnant obese women show a series of complications mainly in the third trimester, such as hypertension, preeclampsia, gestational diabetes, urinary tract infection, thromboembolism, operative vaginal deliveries caesarean-section delivery, anaesthetic and surgical complications, fetal macrosomia, preterm labour and delivery, sudden and unexplained intruterine death, shoulder dystocia, postpartum haemorrhage, postoperative wound infection and endomyometritis in the puerperium (Arabin and Stupin, 2014).

**Management of obesity**

Obesity is the gateway to several issues particularly affecting both male and female fertility, so if one tries to manage obesity; it might be instrumental in management of infertility among obese infertile people. There are various alternatives to manage obesity and metabolic disorder; however, the most effective would be having a drastic change in lifestyle particularly the eating habits. Walking daily for some time is...
considered an effective cardiorespiratory fitness exercise which may drastically improve the metabolic risk profile (Poirier and Després, 2001). Invasive Surgery to reduce weight are effective however, there are reports that such weight loss surgeries are associated with serious complications in 4.1% of patients and a death rate of 0.3%, (Lim et al., 2010).

Non-invasive methods have also been adopted and effective against hyperglycemia to reduce the hepatic glucose through 5’ AMP-activated protein kinase, AMPK activation (Zhou et al., 2001). Although reports exist that these methods might have unacceptable side effects. Glucagon like peptide 1 (GLP-1) analogues which stimulate insulin secretion are used and DPP4-inhibitors that help to prolong GLP-1 action are prescribed without many side effects. Fewer other effective methods are used along with low-calorie intake such as fat absorption blockers, which inhibit the gastric and pancreatic lipases (Curran and Scott, 2004).

New therapeutic methods

There are two main adipose tissue evolved for two different purposes, white adipose tissue (WAT) to survive famine and brown adipose tissue (BAT) to prevent hypothermia (Enerback S, 2010). In addition to these two there is also another intermediate adipose tissue termed beige. Beige adipocytes resemble white adipocytes but possess the classical properties of the brown adipocytes (Whittle et al. 2011; Wu et al. 2012). Studies have quantified BAT oxidative metabolism, glucose and non-esterified fatty acid (NEFA) in 6 healthy human subjects, thus demonstrating unequivocally that BAT contributes to energy expenditure in humans (Ouellet et al., 2012). The presence of the 32 kDa uncoupling protein-1 (UCP-1) in the BAT mitochondria enables heat dissipation thereby resulting in thermogenesis (Nicholls and Locke 1984). During embryonic development, the BAT is formed before other fat depots and is assumed to contain a uniform population of adipocytes. Whereas the beige adipocytes are less clear with respect to its embryonic origin. The browning of the white adipose tissues is often those referred to as “subcutaneous” adipose tissue depots. The depots least able to respond are those often referred to as “visceral”.

Many new therapeutic manipulations of peripheral mechanisms are studied to increase energy expenditure which would be attractive and worthy of focus. Current trends in BAT therapeutics deploy surgically implanting BAT, the augmentation of BAT content and/or enhancement of BAT activity, trans-differentiation of non-BAT progenitors into BAT pre-adipocytes which will increase energy expenditure of obese individuals. Translating the power of BAT into human health appeared much more feasible than reintroducing true BAT into adult men with the concept that certain WAT depots could develop brownish characteristics such as beige, brite, convertible, ectopic, inducible, or recruitable (Schulz et al., 2013). Thus, pragmatically defining browning as any significant increase in UCP1 expression at the mRNA level occurring in white adipose tissue depots (Nedergaard and Cannon, 2014). Numerous transcriptional regulators of brown adipocyte differentiation are currently described in rodents, some revealing promising effects in human models.

A study showed following exercise in both rodents and humans released irisin, a hormone released by muscle, through increased PGC-1α expression. Both through in vitro and in vivo techniques it was showed that irisinhas a powerful browning effect on certain white adipose tissues (Bostrom et al., 2012). Brown adipocyte stem/progenitor cells, CD34+ in skeletal muscle and human multipotent adipose derived stem cells (hMADS) in subcutaneous tissue in adult humans, serve as novel molecular targets for the development of BAT therapeutics. As evidenced that subcutaneous transplantation of embryonic BAT corrected type 1 diabetes in immune-competent mice by reversal of diabetes symptoms, weight regain and normalization of glucose tolerance also that the mice remained euglycaemic 6-months following the procedure (Gunawardana and Piston 2011).

Also, ghrelin, a adipokine, is secreted by the stomach to increase appetite in the fasted state opposing the actions of leptin. Its central administration results in the suppression of the sympathetic activation of BAT thereby reducing energy expenditure and ablation of the ghrelin receptor results in increased BAT thermogenesis and energy expenditure (Mano-Otagiri et al., 2010). Whether other peripheral hormones and adipokines that modulate feeding and energy expenditure would have any effect of thermogenesis requires further investigation.

Conclusion

Currently there is persuasive evidence suggesting targeting brown adipocytes will yield an effective anti-obesity therapy. Finding hormones and adipokine that regulate metabolism and combining novel therapies that enhance BAT activity with an
appetite-suppressant might provide promising and effective management strategies with respect to obesity thereby curbing the associated metabolic disorders highlighted earlier and improving fertility status.

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