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Despite the many advances in biochemical testing, imaging and new therapies, Cushing syndrome (CS) continues to be a challenge. Morbidity and mortality in uncured Cushing patients are high; reports suggesting a 5-year mortality of up to 50% (1). Even in those with cured CS 10-year mortality rates are higher than those without CS (2,3). The key morbidities include opportunistic infection in the short-term and cardiovascular complications in the long-term. A comprehensive strategy of diagnosing urgently, identifying co-morbidities and treating them early, referring to a specialized center appropriately and offering treatment rapidly should decrease the morbidity among patients. It is very appropriate that in this issue of the Journal, the Endocrine Society of Sri Lanka has published the new guidelines on the management of CS. We trust that the clinicians will find clearer directions in managing patients with CS. As in any complex disorder, patients with CS should be comprehensively and rapidly managed with the application of a systematic approach to enable rapid diagnosis leading to early treatment of CS.

Some specialized endocrine centers employ a two-week rule in the management of Cushing syndrome. The two-week rule dictates aggressive investigation to localize the source of Cushing syndrome and, if a diagnosis is not made within a period of two-weeks, the patient is encouraged to undergo bilateral adrenalectomy and testing is continued afterwards. Although not all units are capable of performing all the tests necessary within a short period of time, the principle illustrates the urgency and the life-threatening nature of the illness and is a sober reminder to act with more aggressive intent in the management. It may be preferable in the most severe cases to truncate investigation while medical therapy to lower cortisol levels is undertaken, rather than proceeding to bilateral adrenalectomy in a sick immunocompromised patient who is also at high risk of venous thrombo-embolism. Hypercortisolic patients are immunosuppressed and are susceptible to opportunistic infections, including Aspergillus species, Cryptococcus neoformans, Pneumocystis carinii, Nocardia asteroides, Staphylococcus aureus, Candida albicans, and Herpes simplex (4).

The guidelines by the Endocrine Society of Sri Lanka emphasize the need to employ two screening tests to confirm the diagnosis of Cushing syndrome and early referral to a specialized centre for diagnosing the cause. The initial screening tests either demonstrate disruption of the usual cortisol hemodynamics or an increased cortisol level, and include overnight and low-dose dexamethasone suppression tests, measurement of midnight cortisol and urine free cortisol. In a world where obesity is becoming a common problem, using appropriate clinical skills to select patients who have subtle and early features of Cushing syndrome will be essential. Once Cushing syndrome is diagnosed, measurement of ACTH is used for etiological classification as ACTH-dependent or ACTH-independent Cushing syndrome. Further investigations usually imaging followed by catheter studies and other tests such as CRH or desmopressin stimulation tests would be sufficient in most patients. The difficult cases are the ones where CS caused by a small ectopic lesion that is not immediately apparent.

The treatment of choice is surgery to remove the source of the lesion. A short period of medical therapy to make the patient eucortisolic in order to decrease the metabolic derangements may be necessary before surgery to optimize surgical outcomes, including wound healing. In the patient who is cured or in remission after surgery, continued surveillance is necessary to manage the suppressed pituitary-adrenal axis and to detect recurrence early. One of the key reasons for early mortality in the patient cured of CS is the suppressed hypothalamo-pituitary-adrenal axis: it is imperative to offer the patient appropriate glucocorticoid support necessary for daily living and for emergencies. Long term morbidity and mortality reduction should be achieved by aggressive cardiovascular risk factor control, although data are lacking at the moment to this approach.

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References


Markers of hyperandrogenism in South Asians with polycystic ovary syndrome

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Sri Lanka Journal of Diabetes, Endocrinology and Metabolism 2014; 4: 3-8

Abstract

Background: Many clinical and biochemical criteria are used to assess hyperandrogenism in subjects with polycystic ovary syndrome (PCOS). Standard indicators used to confirm hyperandrogenism are based predominantly on western data. Whilst the phenotype of PCOS has ethnic specific variation, specific cutoffs for hyperandrogenism in South Asians have not been defined.

Objectives: To evaluate the effectiveness of modified Ferriman-Gallwey score (FG Score), serum total testosterone and free androgen index (FAI) in the assessment of hyperandrogenism in PCOS.

Materials and methods: A case control study was conducted on 100 women aged 20-45 years (mean age=30) attending a specialized endocrine clinic in Colombo, Sri Lanka from 1st January 2010 to 1st June 2013. Confirmed cases with PCOS (Rotterdam criteria 2003) were age matched for controls from healthy volunteers.

Recommended cut-offs for hyperandrogenism: FG score ≥8, testosterone (T) >3.5 nmol/L and FAI >5 were applied and receiver operating characteristics (ROC) curves were drawn to compare the diagnostic power of each parameter.

Results: 50 cases with PCOS and 50 controls were studied. Cases versus controls had significantly greater FG score, testosterone (T) and FAI: median FG=10 vs 3, mean testosterone 2.762±1.78 vs 1.045±0.40 (p=0.0001), mean FAI 7.31±7.55 vs 3.64±4.87 (p=0.01); 76% cases and 4% controls had FG score ≥8 (p=0.0001), 30% cases had elevated (T) with none among controls (p=0.00001), 43.3% cases and 14.7% controls had FAI ≥5 (p=0.002). The diagnostic power of serum testosterone was greater than that of FAI in subjects with FG score ≥8. Area under the curve (AUC) for T and FAI were 0.832 and 0.766 respectively.

Conclusion: Clinical assessment by FG score detects hyperandrogenism in PCOS patients more frequently compared to serum testosterone and free androgen index. A higher detection rate was observed in controls when FAI was used as the indicator, suggesting a possible influence from changes in SHBG concentration. Hence, total testosterone having greater diagnostic power than FAI in confirming hyperandrogenism, is the recommended biochemical test in the diagnostic work up of PCOS.

Introduction

The polycystic ovary syndrome (PCOS) is the commonest endocrine disorder of women of reproductive age worldwide (1). A study conducted among a semi-urban population in Sri Lanka has demonstrated that the prevalence of PCOS is 6.3% (2).

According to an editorial published in the British Medical Journal (3), the definition of PCOS by The Androgen Excess and PCOS Society revolves around the presence of hyperandrogenism (clinical and/or biochemical) and ovarian dysfunction (oligo-anovulation) and/or polycystic ovaries with the exclusion of related disorders (4).

Meanwhile the Rotterdam 2003 consensus diagnostic criteria require any 2 out of the 3 criteria to be positive and the criteria being oligo-anovulation, clinical and/or biochemical signs of hyperandrogenism, polycystic ovaries and the exclusion of other aetiologies (congenital adrenal hyperplasia, androgen-secreting tumour, Cushing’s syndrome) (5).

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The commonest cutaneous manifestations of hyperandrogenemia in PCOS include hirsutism, acne and male pattern of hair loss (androgenic alopecia) (6).

In the determination of hyperandrogenism, the true androgen status can be assessed either by measuring free testosterone or by calculating the free androgen index (FAI). The FAI is the ratio of the total serum testosterone (TT) concentration to the concentration of sex hormone binding globulin (SHBG). This is also referred to as the testosterone free index (TFI) and is typically calculated on a molar/molar basis and re-scaled by a factor of ten, one hundred or one thousand, as shown below (7).

\[
FAI = \frac{\text{Total Testosterone (TT) nmol/L}}{\text{SHBG nmol/L}} \times 10, \text{ or } \times 100, \text{ or } \times 1000
\]

The modified Ferriman Gallwey score, serum total testosterone level and FAI are accepted as the markers of hyperandrogenism worldwide. Although there are studies that evaluate the effectiveness of these markers, there is paucity of data in the South Asian context.

Therefore we aimed to evaluate the effectiveness of modified Ferriman-Gallwey score (m-FG Score), serum total testosterone and free androgen index (FAI) in the assessment of hyperandrogenism among South Asians with PCOS.

Methodology

Study design

A case control study was conducted on 100 women aged 20-45 years (mean age = 30 years) attending a specialized endocrine clinic at De Soysa Hospital for Women, Colombo, Sri Lanka from 1st January 2010 to 1st June 2013. Treatment naïve women with PCOS diagnosed by Rotterdam consensus criteria were selected on a convenient sampling basis whilst attending their initial clinic visit at the diagnosis of PCOS. Age matched, unmedicated women among healthy volunteers with regular cycles and no complaints of hyperandrogenism were recruited during the same period as controls. Exclusion of PCOS in controls was confirmed by detailed clinical evaluation, biochemical testing of serum gonadotrophins and testosterone and ovarian ultrasound by a trained radiologist. The control group was also subjected to testing for confounding endocrine disorders such as glucose intolerance and thyroid disease by fasting blood glucose and serum TSH testing.

Subject recruitment

The study subjects were recruited to the two cohorts from the women attending a specialist endocrine clinic at De Soysa Hospital for Women, Colombo, Sri Lanka from 1st January 2010 to 1st June 2013. They were invited to join the study by clinical research associates (MB, SADU, KGHDeS). Volunteers among female staff members from the same institution were recruited after evaluating them and excluding PCOS. Written informed consent was obtained from all cases and controls.

Ethical approval

The project was approved by the Ethics Committee of the Research and Development Divisions of the Faculty of Medicine, University of Colombo, Sri Lanka.

Inclusion criteria

Anovular PCOS: Anovular cycles are defined when the cycle length is more than 35 days, and the lack of demonstrable ovulation by midcycle and luteal phase ultrasound scans, and midluteal serum progesterone.

Polycystic ovaries on ultrasound: defined by transvaginal ultrasound scan of ovaries, performed within the first 5 days from the onset of menstruation, and finding 12 or more follicles, measuring between 2 and 9 mm and/or an ovarian volume >10 cm³ (5).

Hyperandrogenism: Clinical evidence of hirsutism FG ≥8, serum testosterone (T) >3.5 nmol/L and/or FAI >5.

Exclusion criteria

Pregnancy, Cushing’s syndrome, hypothyroidism, hyperprolactinaemia, late onset CAH, androgen secreting ovarian/ adrenal tumour and those on hormonal contraceptives, anti-psychotics, anti-epileptics.

Main outcome measures

Modified Ferriman-Gallwey score (m-FG) was used in women with and without PCOS to quantitatively measure the degree of hirsutism. Serum total testosterone levels and sex hormone binding globulin (SHBG) levels were measured in order to calculate the FAI (FAI = Testosterone (nmol/l) / 100/ SHBG (nmol/l)).

Data collection

Each subject was interviewed by the research associates based on a standard questionnaire that was completed at interview. Menstrual dating and irregularity, hirsutism and acne were recorded.

The presence of hirsutism at baseline clinical evaluation was scored in every woman by the same investigator using the modified FG score and recorded on two occasions with the mean value taken as the final score to quantify the presence of terminal hair over nine body
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areas (i.e., upper lip, chin, chest, upper and lower abdomen, upper and lower back, upper arms and thighs).

Blood samples were collected between 8.00 and 9.00 am within the first 5 days of a spontaneous period. Samples were analyzed at the Reproductive Biology and Endocrinology Laboratory, Department of Obstetrics and Gynaecology, Faculty of Medicine, Colombo, using the following techniques: total testosterone and SHBG – immunometric assay (IMMULITE Diagnostic Products Corporation, USA). Laboratory controls were used to monitor accuracy and precision of the analyzer, reagents and assay results. Inter and intra assay precision checks demonstrated coefficient of variation of 5.4% and 4.3% for testosterone and 3.8% and 3.1% for SHBG.

Data analysis

Calculation and data analysis

Free androgen index = (Testosterone/SHBG) × 100.

Statistical analysis was performed using the computer program Statistical Package for Social Sciences (SPSS version 21.0). The Kolmogorov-Smirnov test was used to determine the normality of distribution for all variables. Continuous data were compared by performing Mann-Whitney test for two medians and categorical data were analyzed by performing the Chi square test.

Diagnostic cut-offs for hyperandrogenism applied for cases and confirmed controls were: FG ≥8, serum testosterone (T) >3.5 nmol/L and FAI >5. The sensitivity and specificity of each of these parameters were determined by using receiver operating characteristics (ROC) curves drawn to compare diagnostic power of each parameter using SPSS. ROC curves were constructed by plotting the sensitivity on the ordinate as a function of the complement of specificity for all the possible cut off values.

Results

50 women with PCOS (mean age 29.4 years) and 50 age matched controls were studied. Table 1 depicts the comparison of clinical and biochemical markers of hyperandrogenism of cases and controls.

Women with PCOS had significant hirsutism when compared with age matched controls. Their mean serum testosterone and FAI mirror this difference. FG score >8 was significantly higher in cases than among age matched controls (p = 0.001); with 76% of women with PCOS vs 4% of controls fulfilling this criterion. Serum testosterone exceeded 3.5 nmol/L in 30% of cases compared to none among controls (p = 0.001). FAI exceeded 5 in 43.3% of cases vs 14.7% controls (p = 0.02).

The ROCs of FAI and testosterone levels are represented in Figure 1. According to the defined cut off the best combination of sensitivity for testosterone is 27% with specificity of 97.5%. The sensitivity at defined cut-off for FAI is 50% with specificity of 87.5%.

The AURCs for FAI and serum testosterone are indicated in Table 2, with a higher value for serum testosterone (0.823) than that for FAI (0.766), which confirms that serum testosterone testing yields a greater probability than FAI in the detection of PCOS.

Table 1. Indicators of hyperandrogenism among south Asian women with PCOS Vs controls

<table>
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<th>Cases</th>
<th>Controls</th>
<th>P value</th>
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<tr>
<td>Number of subjects</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>29.4±5.6</td>
<td>29.6±5.9</td>
<td></td>
</tr>
<tr>
<td>Median FG</td>
<td>10</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Mean serum testosterone (nmol/L)</td>
<td>2.762±1.78</td>
<td>1.045±0.40</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean FAI</td>
<td>7.31±7.55</td>
<td>3.64±4.87</td>
<td>0.01</td>
</tr>
<tr>
<td>FG score &gt; 8</td>
<td>76%</td>
<td>4%</td>
<td>0.0001</td>
</tr>
<tr>
<td>Elevated testosterone (&gt;3.5 nmol/L)</td>
<td>30%</td>
<td>0</td>
<td>0.0001</td>
</tr>
<tr>
<td>FAI &gt; 5</td>
<td>43.3%</td>
<td>14.7%</td>
<td>0.002</td>
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</table>
In 1961, Ferriman and Gallwey described a scoring system to determine the degree of hirsutism using 11 different body sites and this was subsequently subjected to several modifications resulting in a more sensitive, precise version. The modified Ferriman-Gallwey (m-FG) scoring system accepts a total score of equal to or more than 8 as hirsutism from 9 sites (8). This continues to be the most widely used method for visually scoring excess terminal body or facial hair growth for the clinical or investigational assessment of hirsutism (upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, upper arms, and thighs).

Many studies have previously evaluated the effectiveness of the m-FG Score, serum total testosterone and the FAI in determining hyperandrogenism. Ethnic specific differences in the phenotype of PCOS are well known. To the best of our knowledge there has been no report on this aspect of study in the diagnosis of PCOS among indigenous South Asian women.

In vivo and in vitro studies using cultured theca cells consistently showed that ovarian theca cells in affected women with PCOS are more efficient at converting androgenic precursors to testosterone than are normal theca cells (9). Insulin plays both direct and indirect roles in the pathogenesis of hyperandrogenemia in PCOS (9). Nearly a half of the circulating testosterone in normal adult women is derived from the peripheral conversion of androstenedione, and the remainder derived from the ovary and adrenal cortex. The important tissues in which this conversion takes place are the lung, liver, adipose tissue and skin. Plasma dihydrotestosterone is produced virtually entirely by 5 alpha reductase activity in the periphery, with plasma androstenedione being its major precursor. Insulin stimulates testosterone biosynthesis by human theca cells from women with PCOS by activating its own receptor and using inositol glycan mediators as the signal transduction system as well as influencing the FAI through inhibition of hepatic production of SHBG (10).

The Endocrine Society’s Clinical Guideline recommends measuring serum testosterone as a good initial test for hyperandrogenism in the hirsute woman, provided it is less costly and more widely available (11). A case control study evaluated the effectiveness of serum testosterone in the diagnosis of hyperandrogenism among clinically diagnosed patients (n=133; mean age 28 years) with PCOS and healthy volunteers (n=54; mean age 28 years). This study measured total testosterone, SHBG, luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione, dehydroepiandrosterone sulfate (DHEAS) and calculated the bioavailable testosterone by FAI. They found bioavailable testosterone to have a greater accuracy than the FAI, followed by free testosterone (12).

Yet another case control study conducted in the United Kingdom utilized liquid chromatography and tandem mass spectrometry methods for analyzing testosterone and androstenedione in PCOS. The incidence of PCOS being 13.9%, the reference interval for testosterone was 1.8 nmol/l with the conclusion that early follicular phase serum testosterone measured using tandem mass spectrometry and FAI are valuable in the laboratory diagnosis of PCOS (13).

Similar studies performed in Turkey (14) and Oman (15), have concluded that total testosterone and AFI are effective in diagnosing hyperandrogenism, although they reported the AFI being the superior test.

Sri Lanka has major resource limitations with biochemical tests in endocrinology, particularly hyperandrogenism, with prohibitive costs when performing tests such as serum SHBG. However, in order to maintain quality of care in our standard practice when managing the commonest endocrine disorder of young women, there must be an evidence based approach to clinical practice recommendations.
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Hence this study that aims to determine the predictive capability of markers of hyperandrogenism will be useful. The more deviated towards the left upper corner the ROC curve is, the higher the sensitivity and specificity of the diagnostic test for the detection of PCOS. This study shows that total testosterone level has a higher accuracy in the detection of PCOS than the FAI among Sri Lankan women (Figure 1).

The area under ROC curve (AURC) represents the probability of correctly distinguishing between affected and unaffected subjects. Therefore the perfect diagnostic test, not having false positive or false negative results would have an AURC of 1 and on the contrary, a test with AURC ≤ 0.5 would not discriminate affected from non-affected individuals.

This study provides evidence to prove the effectiveness of total testosterone and FAI in South Asians to diagnose hyperandrogenism.

Most studies have found the FAI as a better indicator than serum testosterone to detect hyperandrogenism, while others proved equal effectiveness of both methods, but favouring total testosterone over FAI. Another study conducted in a similar manner has concluded that bioavailable testosterone is more reliable than the FAI (16).

A literature review conducted in 2010 has concluded that the m-FG scoring method is a useful visual instrument for assessing excess terminal hair growth, and the presence of hirsutism, in women (17). This study also provided us with statistically significant evidence for the effectiveness of m-FG to assess hyperandrogenism.

The identified major limitation of the m-FG as an assessment tool is inter-observer variability (18). In the current study the particular bias was avoided by the same investigator performing the physical examination on two separate occasions to determine the m-FG score. Modified FG score is recognized as a clinically useful assessment tool and many studies have suggested that there should be a population specific cut-off value to use in the clinical context (18). The results of this study illustrates that the cut-off point of 8 is appropriate for the South Asian population as well.

Conclusion

Modified Ferriman-Gallwey score (m-FG Score), serum total testosterone and free androgen index (FAI) are effective in the assessment of hyperandrogenism in South Asians. The clinical assessment by FG score detects hyperandrogenism in PCOS patients more frequently compared to serum testosterone and free androgen index. A higher detection rate was observed in controls when FAI was used as the indicator, suggesting a possible influence from changes in SHBG concentration. Our data provides clear evidence to support the measurement of serum total testosterone as the first test when determining hyperandrogenism to confirm the diagnosis of PCOS.

Acknowledgements

We acknowledge with gratitude the Special Trustees, Leeds General Infirmary, UK for providing sustained financial assistance to carry out this study long term and National Research Council (Grant No. 2006/05) of Sri Lanka for providing financial support for the ultrasound scanner. We thank all staff of the Professorial Obstetrics and Gynaecology Unit and Professorial Unit of De Soysa Hospital for Women, Colombo for their ready assistance, support with ultrasound scanning, all staff of the Reproductive Biology and Endocrinology Laboratory, at the Department of Obstetrics and Gynecology, Faculty of Medicine and the patients and their families for their wonderful cooperation.

References


Metabolic syndrome and insulin resistance in an urban and rural adult population in Sri Lanka

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Abstract

Background: To assess the prevalence of metabolic syndrome and insulin resistance in an urban and rural population in Sri Lanka.

Methods: A cross sectional study conducted in 3 health areas selected randomly, one in rural and 2 in urban. In each sector, 180 adults between 30-59 years equally from both sexes were included. Blood pressure, weight, height and waist circumference were measured in each participant. Fasting blood samples were taken to assess plasma glucose (FPG), triglyceride (TG), high density lipoprotein (HDL), insulin and HbA1C. Insulin resistance was detected by the homeostasis model assessment method (HOMA-IR).

Results: From total 345 participants, 46.1% were men and 52.9% women. Prevalence of metabolic syndrome was 25.8% (23.9% in men and 27.4% in women; P = 0.27). Prevalence of each component of MetS in studied population was: 62.9% for central obesity, 28.1% for high TG, 35.9% for low HDL, 40% for high BP, 18.8% for high FPG, 3.9% for high HbA1C, 44.1% for overweight and 9.3% for obesity. Hypeinsulinaemia and insulin resistance was 26.9% and 22.3% respectively. Age, high BMI, waist circumference, FPG, TG, BP, HbA1C, insulin and low HDL significantly higher with subjects with MetS (P = 0.000). Hyperinsulinaemia and insulin resistance was significantly higher in rural sector, obese, centrally obese, high BP and high TG subjects.

Conclusion: One fourth of study population had metabolic syndrome, hyperinsulinaemia and insulin resistance. Metabolic syndrome was strongly associated with hyperinsulinaemia and insulin resistance. Need urgent action to reduce risk in developing type-2 diabetes and cardiovascular diseases in this population.

Keywords: metabolic syndrome, insulin resistance, hyperinsulinaemia

Introduction

Metabolic syndrome (MetS) is a complex disorder with high socioeconomic cost that is considered a worldwide epidemic. The metabolic syndrome is a cluster of the most dangerous heart attack risk factors: diabetes and raised fasting plasma glucose, abdominal obesity, high cholesterol and high blood pressure (1-3). Although there are various definitions for metabolic syndrome, recent review concluded that the definition incorporating International Diabetes Federation (IDF) and American Heart Association / National Heart, Lung and Blood Institute (AHA / NHLBI) is the most suitable for practical use in clinical medicine (1).

People with metabolic syndrome are twice as likely to die from and three times as likely to have heart attack or stroke and have a fivefold greater risk of developing type 2 diabetes compared with people without the syndrome (4). In addition, there is a five fold greater risk of developing type 2 diabetes. MetS would add to the 230 million people worldwide who already have diabetes, one of the most common chronic diseases worldwide and the fourth or fifth leading cause of death in the developed world. The clustering of cardiovascular disease (CVD) risk factors that typifies the metabolic syndrome is now considered to be the driving force for a new CVD epidemic.

Insulin resistance is associated with multiple risk factors for atherosclerosis, including hypertension, dyslipidemia, and glucose intolerance or type 2 diabetes mellitus. Clustering of these risk factors in subjects with

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Obesity has been called “syndrome X” (5), “deadly quartette” (6), “insulin resistance syndrome” (7), or “visceral fat obesity syndrome” (8). Hyperinsulinemia is a good marker of insulin resistance in subjects without significant hyperglycemia and is used for epidemiologic studies.

Insulin resistance and central obesity are considered as significant underlying factors of MetS, which predisposes the global epidemics of type 2 diabetes and cardiovascular diseases (CVD). Genetics, physical activity, ageing, a pro-inflammatory state and hormonal changes may also have a causal effect, but the role may depend on ethnicity (9).

It is estimated that around 20-25 percent of the world’s adult population have metabolic syndrome (4). Prevalence of metabolic syndrome has been reported to be between 12.8% to 41.1% in different parts of the world (10,11). Studies in Sri Lanka showed that the prevalence of metabolic syndrome was between 27.1% to 29.5% (12,13). Many factors including: female gender, increasing age, urban living, higher socio-economic status and physical inactivity were reported as important associated factors (12). According to the World Health Organisation, Non Communicable Diseases (NCDs) are estimated to account for 65% of all deaths in Sri Lanka, accounting 30% of CVD and 4% of diabetes. About 39%, 9%, 22% and 5% of adults are suffering from raised blood pressure, raised blood sugar, overweight and obesity respectively (14).

However, early identification of patients and treatment with appropriate medical and educational programs can be an effective step in controlling and reducing the incidence of metabolic syndrome and in turn cardiovascular diseases and diabetes. In Sri Lanka, last national survey was conducted in 2005-2006. The aim of this study was to assess the recent prevalence of MetS and insulin resistance in an urban and rural population in Sri Lanka to implement suitable interventions to prevent development of diabetes and cardiovascular diseases.

**Materials and methods**

The study population was healthy adults (both male and female) between 30-59 years. Calculated sample size was 360;180 from each urban and rural sector considering 27% of the prevalence of MetS, 95% of precision, 1.1 design effect and 10% non-response rate. A total of 360 adults; 180 from each sector were included in the study. Three Medical Officer of Health (MOH) areas were selected from the Western Province; 2 urban and 1 rural. Ten Public Health Midwives (PHM) were randomly selected from each MOH area to draw the sample. Each selected PHM in the urban MOH areas and rural MOH areas were asked to recruit 9 and 18 healthy adults respectively within the PHM area, starting from randomly selected one starting point, equal numbers from male and female, from each age groups (30-39, 40-49 and 50-59). All the selected adults were asked to attend the relevant MOH office on the predetermined date. Body weight, height and waist circumference were checked. Body mass index (BMI) was estimated as weight in kilograms/height in meters (2). BMI was categorized using both WHO (15) and Asian (16) cut-off levels for comparison purposes. Blood pressure (BP) was measured by a standardized automated measurement system after the participant had been seated for 5 minutes. The mean of 2 readings was used for analysis.

After 12 hour of fasting, blood samples were taken in the morning. All blood samples were labeled and dispatched to the MRI laboratory and samples were centrifuged. Then serum samples were frozen without preservative at ≤20°C and sent to the laboratory of Mickagowa University, Japan for analysis in dry ice. Triglyceride (TG), fasting plasma sugar (FPG), high density lipoprotein (HDL), HbA1C and plasma insulin level were measured using an enzymatic colorimetric method with kits. New International Diabetes Federation definition was used to define MetS for this study (17). Metabolic syndrome was defined as presence of central obesity (defined as waist circumference ≥ 90 cm for male and ≥80 cm for female) plus any two of the following four factors. High triglycerides – TG ≥150 mg/dl; low HDL cholesterol – HDL <40 mg/dl in males and <50 mg/dl in females; high blood pressure – BP systolic ≥130 mmHg or BP diastolic ≥85 mmHg; high fasting plasma glucose – FPG ≥100 mg/dl.

Hyperinsulinaemia was defined as fasting plasma insulin level ≥ 10µIU/MI (18). Overweight, obesity, and high HbA1C was defined as BMI ≥25.0, BMI ≥ 30.0 (15) and ≥ 6.5% respectively (19). Insulin resistance was assessed using the homeostasis model assessment (HOMA-IR) originally described by Mathew et al (20). HOMA-IR was calculated using the following formula: HOMA-IR = (fasting insulin (µIU/ml) / 22.5. Insulin resistance was defined as HOMA-IR >2.5.

Study period was between February to June 2012. Ethical clearance was obtained from the ethical committee of Medical Research Institute. Differences were tested by using analysis of variance and chi square test; significance values were taken at 0.05 level. All data were analyzed by using SPSS for Windows, version 16.0 (SPSS Inc, Chicago).

**Results**

A total 345 study subjects were included in the study and response rate was 95.8%. From total participants, 159 (46.1%) were men and 186 (53.9%) women. Table 1 shows
that the prevalence of metabolic syndrome was 25.8% and there was no significant difference between men and women (23.9% in men and 27.4% in women; P=0.27). It showed significantly increased prevalence of metabolic syndrome with increasing age in total sample, and women. The highest prevalence of metabolic syndrome was seen in age group 50-59 years in total sample, in men and women (35%, 29% and 41% respectively).

Prevalence of each component of MetS in studied population was: 62.9% for central obesity, 28.1% for high TG level, 35.9% for low HDL, 40% for high BP, 18.8% for high FPG, 3.9% for high HbA1C, 26.9% for high insulin, 44.1% for overweight and 9.3% for obesity (Table 2). It showed increased prevalence of central obesity with increasing age. The prevalence of central obesity was significantly higher in women than in men (77.4% vs 45.9%; P=0.000). Central obesity showed highest prevalence in age group 50-59 years in total sample, in men and women (68.3%, 51.6% and 85.2% respectively). High TG and high FPG was significantly more prevalent in men than women (37.1% vs 20.4%; P=0.000, and 27% vs 11.8%; P=0.000 respectively). Conversely low HDL, overweight and obesity was significantly higher in women than men (44.6% vs 25.8%; P=0.000, 50.5% vs 36.5%; P=0.006 and 13.4% vs 4.4%; P=0.003 respectively). The highest prevalence of high BP, high FPG and high insulin was seen in age group of 50-59.

In total study subjects, 30.4% were normal (none of the MetS components were present), 30.1% had one component, 18% two, 6.4% three, 2.9% four and 12.3% had five components of metabolic syndrome (Table 3).

Factors that can affect the prevalence of MetS are shown in Table 4. Among these factors, age, BMI, waist circumference, systolic BP, diastolic BP, FPG, HDL, TG, insulin, HbA1C and HOMA-IR were significantly higher in study subjects with MetS than without (P = 0.00). Sex, area of residence and LDL level showed no significant differences between subjects with MetS and without MetS (P > 0.05).

### Table 1. Prevalence of metabolic syndrome according to sex and age group

<table>
<thead>
<tr>
<th>Age groups in years</th>
<th>N</th>
<th>No. of MetS (%)</th>
<th>Confidence interval (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39</td>
<td>16</td>
<td>15.1</td>
<td>11.3-18.9</td>
</tr>
<tr>
<td>40-49</td>
<td>30</td>
<td>25.9</td>
<td>21.3-30.5</td>
</tr>
<tr>
<td>50-59</td>
<td>43</td>
<td>35.0</td>
<td>29.9-40.0</td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>25.8</td>
<td>21.2-30.4</td>
</tr>
</tbody>
</table>

**Test Statistics**

\( \chi^2 = 10.5; \ P = 0.005 \)

<table>
<thead>
<tr>
<th>Age groups in years</th>
<th>N</th>
<th>No. of MetS (%)</th>
<th>Confidence interval (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td>8</td>
<td>16.0</td>
<td>10.3-21.7</td>
</tr>
<tr>
<td>40-49</td>
<td>12</td>
<td>25.5</td>
<td>18.7-32.3</td>
</tr>
<tr>
<td>50-59</td>
<td>18</td>
<td>29.0</td>
<td>21.9-36.1</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>23.9(^1)</td>
<td>17.3-30.5</td>
</tr>
</tbody>
</table>

**Test Statistics**

\( \chi^2 = 2.9; \ P = 0.23 \)

<table>
<thead>
<tr>
<th>Age groups in years</th>
<th>N</th>
<th>No. of MetS (%)</th>
<th>Confidence interval (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td>8</td>
<td>14.3</td>
<td>9.3-19.3</td>
</tr>
<tr>
<td>40-49</td>
<td>18</td>
<td>26.1</td>
<td>19.8-32.4</td>
</tr>
<tr>
<td>50-59</td>
<td>25</td>
<td>41.0</td>
<td>33.9-48.1</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>27.4(^1)</td>
<td>20.9-33.8</td>
</tr>
</tbody>
</table>

**Test Statistics**

\( \chi^2 = 9.2; \ P = 0.01 \)

\( \chi^2 = 0.5; \ P = 0.27 \)\(^1\)

*Vol.4, No.1, February 2014*
Table 2. Prevalence of each MetS component according to age and sex

<table>
<thead>
<tr>
<th>Age</th>
<th>Central obesity</th>
<th>High TG</th>
<th>Low HDL</th>
<th>High BP</th>
<th>High FPG</th>
<th>High HbA1C</th>
<th>High insulin</th>
<th>Overweight</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>In years</td>
<td>No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td>57 (53.8)</td>
<td>28 (26.4)</td>
<td>37 (34.9)</td>
<td>20 (18.9)</td>
<td>9 (8.5)</td>
<td>3 (3.6)</td>
<td>27 (25.7)</td>
<td>42 (39.6)</td>
<td>9 (8.5)</td>
</tr>
<tr>
<td>40-49</td>
<td>76 (65.5)</td>
<td>33 (28.4)</td>
<td>46 (39.7)</td>
<td>48 (41.4)</td>
<td>24 (20.7)</td>
<td>4 (4.2)</td>
<td>27 (23.7)</td>
<td>52 (44.8)</td>
<td>12 (10.3)</td>
</tr>
<tr>
<td>50-59</td>
<td>84 (68.3)</td>
<td>36 (29.3)</td>
<td>41 (33.3)</td>
<td>70 (56.9)</td>
<td>32 (26.0)</td>
<td>4 (4.0)</td>
<td>38 (30.9)</td>
<td>58 (47.2)</td>
<td>11 (8.9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P values</th>
<th>$\chi^2$=5.6</th>
<th>$\chi^2$=0.24</th>
<th>$\chi^2$=1.1</th>
<th>$\chi^2$=34.5</th>
<th>$\chi^2$=11.8</th>
<th>$\chi^2$=0.01</th>
<th>$\chi^2$=1.7</th>
<th>$\chi^2$=1.4</th>
<th>$\chi^2$=0.3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P=0.06</td>
<td>P=0.89</td>
<td>P=0.56</td>
<td>P=0.000</td>
<td>P=0.003</td>
<td>P=0.6</td>
<td>P=0.4</td>
<td>P=0.5</td>
<td>P=0.9</td>
</tr>
</tbody>
</table>

Sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td></td>
<td>No. (%)</td>
</tr>
<tr>
<td>30-39</td>
<td>73 (45.9)</td>
<td>144 (77.4)</td>
<td>217 (62.9)</td>
</tr>
<tr>
<td>40-49</td>
<td>144 (77.4)</td>
<td>54 (20.3)</td>
<td>198 (72.1)</td>
</tr>
<tr>
<td>50-59</td>
<td>52 (29.3)</td>
<td>15 (7.4)</td>
<td>67 (27.8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P values</th>
<th>$\chi^2$=36.5</th>
<th>$\chi^2$=11.7</th>
<th>$\chi^2$=13.2</th>
<th>$\chi^2$=0.9</th>
<th>$\chi^2$=12.9</th>
<th>$\chi^2$=0.00</th>
<th>$\chi^2$=0.4</th>
<th>$\chi^2$=6.8</th>
<th>$\chi^2$=8.3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P=0.000</td>
<td>P=0.000</td>
<td>P=0.000</td>
<td>P=0.4</td>
<td>P=0.000</td>
<td>P=0.6</td>
<td>P=0.3</td>
<td>P=0.006</td>
<td>P=0.003</td>
</tr>
</tbody>
</table>

| Test statistics |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Male            | $\chi^2$=2.1 | $\chi^2$=1.0 | $\chi^2$=1.4 | $\chi^2$=6.9 | $\chi^2$=8.5 | $\chi^2$=0.04 | $\chi^2$=2.1 | $\chi^2$=0.6 | $\chi^2$=1.2 |
|                 | P=0.35       | P=0.59       | P=0.5         | P=0.03       | P=0.01       | P=0.9          | P=0.4          | P=0.7         | P=0.5         |

| Female          | $\chi^2$=2.6 | $\chi^2$=1.8 | $\chi^2$=3.24 | $\chi^2$=3.6 | $\chi^2$=1.1 | $\chi^2$=3.1 | $\chi^2$=1.2 | $\chi^2$=0.6 |
|                 | P=0.08       | P=0.28       | P=0.9         | P=0.000      | P=0.2        | P=0.58        | P=0.2         | P=0.5         | P=0.7         |

(Overweight = BMI ≥ 25.0 and Obesity = BMI ≥ 30.0)
Table 3. Prevalence of metabolic syndrome components and insulin resistance in studied population

<table>
<thead>
<tr>
<th>No. of components</th>
<th>No.</th>
<th>%</th>
<th>Confidence interval (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No any component</td>
<td>105</td>
<td>30.4</td>
<td>25.6-35.3</td>
</tr>
<tr>
<td>1 component</td>
<td>104</td>
<td>30.1</td>
<td>25.3-34.9</td>
</tr>
<tr>
<td>2 components</td>
<td>62</td>
<td>18.0</td>
<td>14.0-22.1</td>
</tr>
<tr>
<td>3 components</td>
<td>22</td>
<td>6.4</td>
<td>3.8-8.9</td>
</tr>
<tr>
<td>4 components</td>
<td>10</td>
<td>2.9</td>
<td>1.1-4.7</td>
</tr>
<tr>
<td>5 components</td>
<td>42</td>
<td>12.3</td>
<td>8.8-15.8</td>
</tr>
<tr>
<td>Insulin resistance (HOMA-IR)</td>
<td>342</td>
<td>22.3</td>
<td>19.8-28.9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>345</strong></td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Relationship of MetS with associated factors

<table>
<thead>
<tr>
<th>Associated factors</th>
<th>No. (%) with MetS</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Age groups in years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td>16</td>
<td>90</td>
<td>106</td>
</tr>
<tr>
<td>40-49</td>
<td>30</td>
<td>86</td>
<td>116</td>
</tr>
<tr>
<td>50-59</td>
<td>43</td>
<td>80</td>
<td>123</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>38</td>
<td>121</td>
<td>159</td>
</tr>
<tr>
<td>Female</td>
<td>51</td>
<td>135</td>
<td>186</td>
</tr>
<tr>
<td>Area of residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>41</td>
<td>135</td>
<td>176</td>
</tr>
<tr>
<td>Rural</td>
<td>48</td>
<td>121</td>
<td>169</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>1</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>18.5-24.9</td>
<td>30</td>
<td>140</td>
<td>170</td>
</tr>
<tr>
<td>25.0-29.9</td>
<td>44</td>
<td>76</td>
<td>120</td>
</tr>
<tr>
<td>≥30.0</td>
<td>14</td>
<td>19</td>
<td>33</td>
</tr>
<tr>
<td>BMI Asian cut-off values (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>1</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>18.5-22.9</td>
<td>16</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>23.0-24.9</td>
<td>14</td>
<td>56</td>
<td>70</td>
</tr>
<tr>
<td>≥ 25.0</td>
<td>58</td>
<td>95</td>
<td>153</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>89 (25.8)</strong></td>
<td><strong>256 (74.2)</strong></td>
<td><strong>345</strong></td>
</tr>
</tbody>
</table>

Mean (SD)

<table>
<thead>
<tr>
<th></th>
<th>F=;P=0.000</th>
<th>F=;P=0.000</th>
<th>F=;P=0.000</th>
<th>F=;P=0.000</th>
<th>F=;P=0.000</th>
<th>F=;P=0.000</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>26.4 (3.9)</td>
<td>23.9 (4.0)</td>
<td>24.6 (4.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>94.2 (7.9)</td>
<td>85.3 (10.2)</td>
<td>87.6 (10.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPG (mg/l)</td>
<td>102.6 (28.7)</td>
<td>91.7 (22.9)</td>
<td>94.5 (24.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG (mg/l) 170.4 (73.6)</td>
<td>110.1 (61.5)</td>
<td>125.6 (69.9)</td>
<td></td>
<td>F=;P=0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mg/l)</td>
<td>44.5 (10.0)</td>
<td>50.3 (10.1)</td>
<td>48.8 (10.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL (mg/l)</td>
<td>153.5 (37.9)</td>
<td>144.6 (35.9)</td>
<td>146.9 (36.5)</td>
<td></td>
<td>F=;P=0.05</td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>136.8 (14.6)</td>
<td>121.8 (15.9)</td>
<td>125.7 (16.9)</td>
<td></td>
<td>F=;P=0.000</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>81.1 (11.0)</td>
<td>72.1 (11.0)</td>
<td>74.4 (11.7)</td>
<td></td>
<td>F=;P=0.000</td>
<td></td>
</tr>
<tr>
<td>HbA1C level (%) (n=279)</td>
<td>4.3 (1.4)</td>
<td>3.9 (1.2)</td>
<td>4.0 (1.2)</td>
<td></td>
<td></td>
<td>F=;P=0.01</td>
</tr>
<tr>
<td>Plasma insulin level (μIU/mL)</td>
<td>10.7 (10.5)</td>
<td>6.9 (4.4)</td>
<td>7.9 (6.7)</td>
<td></td>
<td>F=;P=0.000</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR (n=342)</td>
<td>2.72 (2.8)</td>
<td>1.65 (1.9)</td>
<td>1.93 (2.2)</td>
<td></td>
<td></td>
<td>F=;P=0.000</td>
</tr>
</tbody>
</table>
Table 5 shows that prevalence of hyperinsulinemia was 26.9% and no significant difference between men and women (25.3% in men and 28.3% in women). The highest prevalence of hyperinsulinemia was seen in age group 50-59 years (30.9%). The prevalence of hyperinsulinemia was significantly higher in study subjects living in rural sector (33.1% vs 21%; P=0.01), having metabolic syndrome (46.6% vs 20.1%; P=0.000), central obesity (33% vs 16.5%; P=0.001), high BP (26.2% vs 20.6%; P=0.001), low HDL (34.7% vs 22.6%; P=0.01) and high TG (34.4% vs 24%; P=0.04). The highest prevalence of hyperinsulinaemia was seen in obese subjects according to both WHO and Asian classification (42.4% and 36.8% respectively).

Prevalence of insulin resistance was 22.3% and significantly higher in study subjects with metabolic syndrome (44.3% vs 14.6%; P=0.000), obesity (33.3% vs 31.1% and 16.1%; P=0.002), central obesity (26.5% vs 15.1%; P=0.009), high BP (30.7% vs 16.7%; P=0.002) and high TG (33.3% vs 18%; P=0.002) as shown in Table 5.

Table 5. Prevalence of hyperinsulinaemia and insulin resistance in MetS associated factors

<table>
<thead>
<tr>
<th>Associated factors</th>
<th>Total</th>
<th>Hyperinsulinaemia</th>
<th>HOMA-LR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>95% CI</td>
<td>P value</td>
</tr>
<tr>
<td><strong>Age groups in years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td>105</td>
<td>27 (25.7)</td>
<td>21.7-30.3</td>
</tr>
<tr>
<td>40-49</td>
<td>114</td>
<td>27 (23.7)</td>
<td>19.2-28.2</td>
</tr>
<tr>
<td>50-59</td>
<td>123</td>
<td>38 (30.9)</td>
<td>26.0-35.8</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>158</td>
<td>40 (25.3)</td>
<td>20.7-29.9</td>
</tr>
<tr>
<td>Female</td>
<td>184</td>
<td>52 (28.3)</td>
<td>23.5-33.1</td>
</tr>
<tr>
<td><strong>Area of residence</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>176</td>
<td>37 (21.0)</td>
<td>16.7-25.3</td>
</tr>
<tr>
<td>Rural</td>
<td>166</td>
<td>55 (33.1)</td>
<td>28.1-38.1</td>
</tr>
<tr>
<td><strong>Metabolic syndrome</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>88</td>
<td>41 (46.6)</td>
<td>41.3-51.9</td>
</tr>
<tr>
<td>No</td>
<td>254</td>
<td>51 (20.1)</td>
<td>15.9-24.4</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>21</td>
<td>1 (4.8)</td>
<td>2.5-7.1</td>
</tr>
<tr>
<td>18.5-24.9</td>
<td>169</td>
<td>35 (20.7)</td>
<td>16.4-24.9</td>
</tr>
<tr>
<td>25.0-29.9</td>
<td>119</td>
<td>42 (35.3)</td>
<td>30.2-40.4</td>
</tr>
<tr>
<td>≥30.0</td>
<td>33</td>
<td>14 (42.4)</td>
<td>37.2-47.6</td>
</tr>
<tr>
<td><strong>BMI Asian cut-off</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>21</td>
<td>1 (4.8)</td>
<td>2.5-7.1</td>
</tr>
<tr>
<td>18.5-22.9</td>
<td>99</td>
<td>17 (17.2)</td>
<td>13.2-21.2</td>
</tr>
<tr>
<td>23.0-24.9</td>
<td>70</td>
<td>18 (25.7)</td>
<td>21.1-30.3</td>
</tr>
<tr>
<td>≥25.0</td>
<td>152</td>
<td>56 (36.8)</td>
<td>31.7-41.9</td>
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<td><strong>Central obesity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>215</td>
<td>71 (33.0)</td>
<td>28.0-37.9</td>
</tr>
<tr>
<td>No</td>
<td>127</td>
<td>21 (16.5)</td>
<td>12.6-20.4</td>
</tr>
<tr>
<td><strong>High BP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>138</td>
<td>50 (26.2)</td>
<td>31.1-41.3</td>
</tr>
<tr>
<td>No</td>
<td>204</td>
<td>42 (20.6)</td>
<td>16.3-24.9</td>
</tr>
<tr>
<td><strong>Low HDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>121</td>
<td>42 (34.7)</td>
<td>29.7-39.7</td>
</tr>
<tr>
<td>No</td>
<td>221</td>
<td>50 (22.6)</td>
<td>18.2-27.0</td>
</tr>
<tr>
<td><strong>High TG</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>96</td>
<td>33 (34.4)</td>
<td>29.4-39.4</td>
</tr>
<tr>
<td>No</td>
<td>246</td>
<td>59 (24.0)</td>
<td>19.5-28.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>342</td>
<td>92 (26.9)</td>
<td>22.2-31.6</td>
</tr>
</tbody>
</table>
Discussion

With the metabolic syndrome driving the twin global epidemics of type 2 diabetes and CVD there is an overwhelming moral, medical and economic imperative to identify those individuals with metabolic syndrome early, so that lifestyle interventions and treatment may prevent the development of diabetes and or CVDs (5). The results of this study showed that the prevalence of metabolic syndrome is high in studied population and it increases with increment of age and BMI significantly. The prevalence of MetS was 25.8% in this study which is lower than previous studies in Sri Lanka (27.1%) (12). This may be due to methodological and sampling differences between these studies. In DECODE study that was conducted in 9 European countries, MetS was detected in 32% of men and 28.5% of women (21). The prevalence of MetS has been reported in other parts of the world with different diagnostic criteria made the comparison difficult.

Prevalence of high BP (40%), high FPG (18.8%), overweight (44.1%) and obesity (9.3%) was higher in our sample than the prevalence estimated by the WHO in 2008 (39.2%, 8.8%, 21.9% and 5.1%) (14). However, high BP was lower (40%) in this study than the previous national study in 2006 (81.1%) (12). Prevalence of high TG in our study was 28.1%; 37.1% in men and 20.4% in women but in previous study it was 55.1% (12), in Sri Lanka. It may be due to different criteria used to define the components of MetS in these studies.

Relationship between associated factors and MetS showed very useful information for future actions. Our study identified that age, BMI, central obesity, FPG, TG, HDL, LDL, systolic BP, diastolic BP, HbA1C, plasma insulin, HOMA-I had significant relationship with MetS, but not with the area of residence. It was identified in some studies that many factors including: age, ethnicity, weight, menopause in women, smoking, low income economies, high carbohydrate intake, and low physical activity play a role in metabolic syndrome (22).

In most studies (23,24) increasing age was the key factor affecting the prevalence of metabolic syndrome and it was also shown in our study. Increasing in BMI was correlated with increasing prevalence of MetS, hyperinsulinaemia and insulin resistance in this study. This is in agreement with other studies (9). In this study, prevalence of metabolic syndrome was significantly higher in women than men as shown in other studies (12,23). This may be due to reduction of physical activity in Sri Lankan women which may cause higher rate of central obesity and low HDL. Strengths of our study was that it is a community based study including both urban and rural population of Sri Lanka. However, there was no difference of prevalence of MetS between urban and rural populations but rural subjects had significantly high level of insulin resistance. One limitation was that, this study was cross sectional that does not allow us to draw any causal interference which indicates the need of a longitudinal study.

Nearly one third of our sample did not have any component of MetS which is lower than the previously reported figure (51%) (13). Presence of two or more components were noted in 27.3% of our sample compared to the previous study (25% between 20-40 years) (13). This information is very useful to make decision on appropriate population screening to prevent MetS in the future.

Prevalence of hyperinsulinaemia in our study was 26.9%, 25.3% in men and 28.3% in women and insulin resistance was 22.3%. Living in rural area, presence of metabolic syndrome, obesity, central obesity, high BP and high TG were strongly associated with both hyperinsulinaemia and insulin resistance.

Studies in the general population revealed that hyperinsulinemia is an early feature, of the cardiovascular clusters of MetS. It a surrogate marker to predict future development of MetS, but also the increased risk for future CVD as well (25). Therefore the results of our study strongly emphasise the urgent need of implementing lifestyle modifications for this population which help in reversing hyperinsulinaemia and insulin resistance to prevent MetS and CVD in the future.

Conclusions

Metabolic syndrome has high prevalence in our population and its prevalence increases with increasing age and BMI. Women are at higher risk for MetS than men. Metabolic syndrome was strongly associated with hyperinsulinaemia and insulin resistance. Strategies to reverse hyperinsulinaemia to reduce the risk in developing type-2 diabetes and cardiovascular diseases need to be identified.

Acknowledgement

We would like to thank medical officers of health and staff of Kotte, Kollonnawa and Aththanagalla for assisting us in collecting data and samples, staff of the Department of Nutrition, Medical Research Institute in collecting data and samples.

References


Premature coronary artery disease and testosterone in Sri Lankan men

C M Wickramatilake¹, M R Mohideen², C Pathirana¹


Abstract

Objective: Recent studies have shown that low endogenous testosterone in men is associated with coronary artery disease (CAD) and its risk factors such as obesity, hypertension, dyslipidaemia, and diabetes mellitus. This study was planned to investigate the association of low total testosterone levels with premature coronary artery disease.

Methods: Thirty one men, 45 years of age or below with documented coronary artery disease (mean age 41 ± 3) constituted the cases. Control group consisted of 31 men below the same age, without having clinically evident coronary artery disease (mean age 37 ± 4). Total testosterone, fasting plasma glucose and serum lipid levels were measured.

Results: Mean ages of the two groups were significantly different (p = 0.001). Body mass index (p = 0.843) and hip circumferences (p = 0.097) were not significantly different between the two groups, but waist circumference (p = 0.007) and waist to hip ratio (p = 0.002) were significantly higher among cases. Prevalence of hypertension, diabetes mellitus, and smoking among cases was higher compared to controls. Total testosterone levels of cases were significantly lower than those of controls (11.1 ± 3.2 nmol/L vs. 27.1 ± 4.3 nmol/L, p = 0.001), which remained significant, following adjustment for the clinical covariates (age, BMI, smoking, diabetes mellitus). Plasma glucose (p = 0.016) and HDL-cholesterol (p = 0.001) were significantly different between the groups.

Conclusion: Serum total testosterone was significantly lower in patients with premature CAD compared to controls. Low level of total testosterone may be related to the development of premature coronary artery disease.

Keywords: premature coronary artery disease; testosterone; men

Introduction

The age-standardized death rates for coronary heart disease are declining in many developed countries, but are increasing in developing countries such as in Sri Lanka with demographic changes, urbanization, and lifestyle changes (1, 2). Coronary artery disease (CAD) is one of the leading causes of mortality in men. Cardiovascular disease accounts for the highest rate of hospital deaths in Sri Lanka (2). Men of similar age tend to have higher incidence of CAD than premenopausal women (3). Hence, it is fair to assume that androgens may have a role in the development of CAD. In contrast, epidemiological studies have identified an inverse association between testosterone and coronary artery disease in men (4, 5, 6) with reduced testosterone concentrations being linked to premature CAD (7). There are studies indicating that a low testosterone level is related to the risk factors of cardiovascular disease as well (8, 9, 10).

Nevertheless, the relationship between testosterone and CAD has not been established yet. Moreover, the relationship between premature CAD and testosterone levels is not well studied. There are no previous studies of this nature conducted in Sri Lanka. Therefore this study was done to investigate whether low total testosterone levels are associated with premature CAD.

Materials and methods

The research project was approved by the Ethical Review Committee of Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka. Thirty one men of 45 years of age or below with documented coronary artery disease constituted the cases. It included 11 with angiogram proven coronary artery disease patients who were awaiting coronary artery bypass graft at Cardiothoracic Unit, Teaching Hospital, Karapitiya and 20 with confirmed ST-
elevated myocardial infarction patients admitted to Coronary Care Unit, Teaching Hospital, Karapitiya. The control group consisted of 31 men below the same age, without having clinically evident coronary artery disease. They were selected from the patients who were awaiting minor surgery at surgical units, Teaching Hospital, Karapitiya.

Data were collected using an interviewer-administered questionnaire, after the informed written consent. Anthropometric measurements were obtained according to the standard protocols. Fasting morning blood samples were collected for biochemical assays. Serum testosterone levels were estimated using commercial enzyme immuno assay kit (Pathozyme Testosterone, Omega Diagnostics Ltd, Omega House, UK). Concentrations of fasting plasma glucose, serum total cholesterol, high-density lipoprotein cholesterol and triglyceride levels were measured by colourimetric methods using commercial kits (ProDia International, UAE).

Data were analyzed using Minitab version 15 for Windows. Categorical data were displayed as percentages and frequencies. Categorical data were analyzed using the Chi-squared test or Fisher’s exact test. Numerical data were examined for normality and presented as mean ± SD. Adjusted means of hormone, lipid levels were calculated using multiple regressions. Age, body mass index, smoking and diabetes were used as independent predictor variables for testosterone and age, BMI, smoking, diabetes and use of statin were used as independent variables for lipids and for plasma glucose; age and BMI were used. Comparison between cases and controls was analyzed using two sample t-tests for independent samples assuming unequal variance. Pearson correlation coefficient was used to assess the relationship between serum testosterone concentrations and other risk factors.

Results

The age of the CAD group ranged from 32 to 45 years and the age of control group varied from 30 to 45 years. Mean ages of the two groups were significantly different (41 ± 3 vs. 37 ± 4 years, p = 0.001). Although BMI (23.6 ± 4 vs. 23.5 ± 6, p = 0.906) and hip circumferences (89.7 ± 8 vs. 86.3 ± 8 cm, p = 0.133) were not significantly different between the two groups, waist circumference (84.3 ± 10 vs. 76.7 ± 11 cm, p = 0.01) and the waist-hip ratio (0.93 ± 0.04 vs. 0.88 ± 0.06, p = 0.001) were significantly different (Table 1).

Prevalence of hypertension, diabetes mellitus and history of statin use among the cases were 8 (25.8 %), 4 (12.9 %), 13 (41.9 %) respectively, while none reported in the control group. Smoking was reported in 23 (74.2 %) cases; 15 of them were current smokers and eight were ex-smokers for approximately three months at the time of recruitment to the study. 6 (19.3 %) controls were past-smokers and they had stopped smoking for more than one year by the time of recruitment to the study (Table 1).

Table 1. Baseline characteristics in cases vs. controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n = 31)</th>
<th>Control group (n = 31)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years)</td>
<td>41 ± 3</td>
<td>37 ± 4</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI (Kgm⁻²)</td>
<td>23.6 ± 4</td>
<td>23.4 ± 6</td>
<td>0.843</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>84.3 ± 10</td>
<td>76.5 ± 11</td>
<td>0.007</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>89.7 ± 8</td>
<td>85.9 ± 8</td>
<td>0.097</td>
</tr>
<tr>
<td>W-H ratio</td>
<td>0.93 ± 0.04</td>
<td>0.88 ± 0.06</td>
<td>0.002</td>
</tr>
<tr>
<td>SBP mmHg</td>
<td>134 ± 28</td>
<td>123 ± 13</td>
<td>0.042</td>
</tr>
<tr>
<td>DBP mmHg</td>
<td>83 ± 18</td>
<td>77 ± 7</td>
<td>0.090</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4 (12.9 %)</td>
<td>Not present</td>
<td>-</td>
</tr>
<tr>
<td>Hypertension</td>
<td>8 (25.8 %)</td>
<td>Not present</td>
<td>-</td>
</tr>
<tr>
<td>Smoking</td>
<td>23 (74.2 %)</td>
<td>6 (18.7 %)</td>
<td>0.001</td>
</tr>
<tr>
<td>Statin treatment</td>
<td>13 (41.9 %)</td>
<td>Not used</td>
<td>-</td>
</tr>
</tbody>
</table>

BMI = Body mass index, W-H ratio = Waist to hip ratio, SBP = Systolic blood pressure, DBP = Diastolic blood pressure. Data presented as mean ± SD, percentages or frequencies, p-Value calculated using two-sample t-test. Other p values calculated using Chi-squared test or Fisher’s exact test.
Serum total testosterone concentration (TT) among the cases was significantly lower than the controls (p = 0.001) which remained significant (p = 0.001) even after adjustments for clinical covariates (age, BMI, smoking, diabetes mellitus).

The concentrations of total cholesterol (T-Ch), LDL-cholesterol (LDL-Ch) and triglycerides (TGs) were not significantly different between the two groups, but after the adjustments for the clinical covariates (age, BMI, smoking, diabetes mellitus and use of statin), the difference between the two groups became significant; T-Ch (p = 0.004), LDL-Ch (p = 0.001), TGs (p = 0.001). Serum HDL-Cholesterol (HDL-Ch) was significantly different between two groups (Table 2). Plasma glucose concentration in the CAD group was significantly higher compared to the controls (p = 0.016), which became stronger (p = 0.001) after the adjusting for age and BMI.

A significant positive correlation was observed between total testosterone and the HDL-Ch (p = 0.001). A negative correlation existed between total testosterone and age (p = 0.001), waist circumference (p = 0.008), W-H ratio (p = 0.003) showed highly significant (all p < 0.05), while modest significance was present between TT and hip circumference (p = 0.087), SBP (p = 0.067), DBP (p = 0.057) and relationship was not significant with BMI (p = 0.869). The negative correlations between TT and TCh (p = 0.308), TGs (p = 0.229) and PG (p = 0.115) were non significant, while modest significance was present with LDL-Ch (p = 0.065).

### Table 2. Comparison of laboratory findings

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Patients (n=31) unadjusted</th>
<th>Controls (n=31) unadjusted</th>
<th>p</th>
<th>Patients (n=31) adjusted*</th>
<th>Controls (n=31) adjusted*</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT (nmol/L)</td>
<td>11.1 ± 3.2</td>
<td>27.1 ± 4.3</td>
<td>0.001</td>
<td>11.1 ± 1.2</td>
<td>27.0 ± 3.2</td>
<td>0.001</td>
</tr>
<tr>
<td>T-Ch (mmol/L)</td>
<td>4.7 ± 2</td>
<td>5.2 ± 1.3</td>
<td>0.34</td>
<td>4.7 ± 0.7</td>
<td>5.2 ± 0.3</td>
<td>0.004</td>
</tr>
<tr>
<td>HDL-Ch (mmol/L)</td>
<td>1.0 ± 0.4</td>
<td>1.7 ± 0.7</td>
<td>0.001</td>
<td>1.0 ± 0.2</td>
<td>1.7 ± 0.3</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL-Ch (mmol/L)</td>
<td>3.7 ± 1.8</td>
<td>3.1 ± 0.4</td>
<td>0.13</td>
<td>3.6 ± 0.7</td>
<td>3.1 ± 0.1</td>
<td>0.001</td>
</tr>
<tr>
<td>TGs (mmol/L)</td>
<td>2.0 ± 1</td>
<td>1.6 ± 0.8</td>
<td>0.11</td>
<td>2.0 ± 0.3</td>
<td>1.6 ± 0.08</td>
<td>0.001</td>
</tr>
<tr>
<td>PG (mmol/L)</td>
<td>6.2 ± 2.5</td>
<td>4.9 ± 0.6</td>
<td>0.016</td>
<td>6.2 ± 0.6</td>
<td>4.9 ± 0.2</td>
<td>0.001</td>
</tr>
</tbody>
</table>

TT = total testosterone, TGs = triglycerides, TCh = total cholesterol, HDL-Ch = high-density lipoprotein cholesterol, LDL-Ch = low density lipoprotein cholesterol, PG = plasma glucose. All values expressed as mean ± SD. p-Values stated calculated by two-sample t-test. * The measurements were adjusted for clinical covariate in the regression analysis.

### Table 3. Relationships between testosterone levels and other measurements

<table>
<thead>
<tr>
<th>Measurements</th>
<th>TT</th>
<th>Measurements</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.593 (0.001)</td>
<td>TGs</td>
<td>-0.155 (0.229)</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.021 (0.869)</td>
<td>T-Ch</td>
<td>-0.132 (0.308)</td>
</tr>
<tr>
<td>Waist</td>
<td>-0.332 (0.008)</td>
<td>HDL-Ch</td>
<td>0.719 (0.001)</td>
</tr>
<tr>
<td>Hip</td>
<td>-0.219 (0.087)</td>
<td>LDL-Ch</td>
<td>-0.236 (0.065)</td>
</tr>
<tr>
<td>W-H ratio</td>
<td>-0.374 (0.003)</td>
<td>PG</td>
<td>-0.202 (0.115)</td>
</tr>
<tr>
<td>SBP</td>
<td>-0.235 (0.067)</td>
<td>DBP</td>
<td>-0.243 (0.057)</td>
</tr>
</tbody>
</table>

Number outside the parentheses represents value of correlation coefficient; p values for correlation coefficient are shown in the parentheses. Abbreviations as in Table 1, 2.
Discussion

The current study reveals that serum total testosterone concentration was significantly lower in patients with premature coronary artery disease compared to the controls. This finding is consistent with the previous studies suggesting that low testosterone may be a risk factor for the development of CAD (4, 5, 6). Furthermore, a recent study done on Turkish subjects has linked the presence of low testosterone to the development of premature CAD, where both total and free testosterone found to be low in the cases compared to controls (7). Findings of our study are in agreement of the above study.

Prevalence of conventional risk factors such as diabetes mellitus, hypertension, hyperlipidaemia and smoking were more prevalent in the premature CAD group. Although BMI was not significantly different between the two groups, WC and W-H ratio were higher in the CAD group reflecting the relationship of central obesity with the development of CAD (8, 9, 10).

HDL-Ch level was significantly higher in the control group compared to cases and also showed a significant positive correlation with testosterone. Total testosterone showed modestly significant negative correlation with LDL-Ch. These findings are in agreement with the previous reports, which had shown positive correlation of testosterone with HDL-Ch in healthy men. One proposed mechanism is that testosterone acts through modulation of lipid profile, supported by findings of a favourable relationship with HDL-Ch (11, 12, 13).

There are studies where intramuscular administration of near-physiological doses of testosterone resulted in a decrease in total and LDL cholesterol levels without significantly affecting HDL cholesterol levels (14-15). Testosterone supplementation in men with hypogonadotropic hypogonadism were shown to increase levels of total and LDL cholesterol, but increased the HDL /LDL ratio (16).

Conclusion

The serum total testosterone was significantly lower in patients with premature coronary artery disease compared to controls. There was a significant positive association between serum total testosterone and HDL-Ch in both groups. This reflected that low levels of testosterone appear to be related to the development of atherogenic lipid profile and premature coronary artery disease. It is recommended that larger studies are needed to confirm these findings in future.

Conflicts of interest: The authors declared that they had no conflicts of interest concerning this article.

Acknowledgement

We wish to acknowledge the University Grants Commission, Sri Lanka for the financial assistance provided for the project, Mrs. DABN Amerasekara, (Statistician, Applied Statistic Association, Sri Lanka), Senior Lecturer, Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka for the statistical advice provided, and Mrs. Kawmadhi Abeywaradana, Technical Officer, Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka for the support offered in the biochemical assays.

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Continuous subcutaneous insulin infusion versus multiple dose insulin

Yashdeep Gupta¹, Sanjay Kalra²


Abstract

Continuous subcutaneous insulin infusion (CSII), also known as insulin pump therapy and multiple dose insulin (MDI) are the two main intensified insulin regimens that have been used to achieve strict glycaemic control in patients with diabetes mellitus, especially with type 1 diabetes (T1DM). Each mode of therapy has its own merits and demerits. CSII is a costly and complex therapeutic intervention, but on the other hand provides flexibility, improves patient’s quality of life, glycaemic control and decreases hypoglycaemic episodes in certain situations. This review will summarize current evidence on CSII vs MDI on various outcomes. This will help health professionals to select the most appropriate method of insulin administration for the given situation.

Keywords: continuous subcutaneous insulin infusion, CSII, insulin pump therapy, multiple dose insulin, MDI, diabetes men

Introduction

Suboptimal glycemic control in individuals with diabetes leads to chronic complications and increases mortality. Therefore, efficient glucose control is essential for the prevention of life threatening complications of the disease. The increasing need of aggressive diabetes treatment has led to the improvement of insulin therapy and its implementation techniques. CSII and MDI regimens are the two main intensified insulin regimens. The development of CSII by insulin pumps and short-acting insulin analogues are innovations designed to improve glycaemic control and quality of life (QOL), while limiting adverse effects, such as hypoglycaemia. They represent important advances in the treatment of diabetes.

CSII, also known as insulin pump therapy, uses a small, portable electromechanical pump to infuse short-acting insulin via a subcutaneously implanted cannula to provide basal delivery, with patient-activated prandial boluses. MDI, also known as basal-bolus therapy, is a regimen that employs long-acting insulin formulations (isophane, glargine, detemir) to supply the basal component, with rapid acting insulin (aspart, lispro, glulisine) or short acting insulin (regular insulin) to supply the boluses.

The insulin pump therapy is an expensive addition in the management of patients with diabetes. Therefore, it is important to understand whether CSII provides additional benefits in terms of glycaemic control, hypoglycaemic episodes, quality of life and certain other parameters as compared to MDI. We summarize available data mainly from latest meta-analysis that compare pumps with multiple dose insulin injections. The review will address the issues separately on type 1 diabetes mellitus in children, type 1 diabetes mellitus in adults, type 2 diabetes mellitus and pregnancy. This will be helpful in providing objective information to health professionals when making decisions about the use of CSII in clinical practice.

Search strategy: Pubmed with “Insulin pump therapy” or “Continuous subcutaneous insulin infusion” or “Insulin analogues”. Limits Meta-analysis, Reviews, Systematic reviews, Randomised control trials, Guidelines, English language, 10 years. Additional relevant cross references were retrieved.

Type 1 diabetes mellitus (adults)

HbA1c

A recent meta-analysis compared rapid-acting analogue-based CSII with MDI (rapid-acting analogues with neutral protamine Hagedorn/long acting analogue insulin) (1). It found that CSII decreased HbA1c levels more than MDI did, combined mean between-group difference was -0.30%. However, the pooled estimate was influenced by 1 study in which participants had a higher HbA1c level at enrolment (9.3%) compared with that of the other studies (7.7% to 8.2%), resulting in greater
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opportunity for a large decrease in HbA1c levels in that study (-0.84%) than in the other studies (-0.1% to 0.25%). The difference between CSII and MDI became null (combined mean between-group difference, -0.01%) after this study was removed. Furthermore, the study with largest effect used NPH insulin as basal insulin (2). The studies which used glargine as basal insulin in addition to rapid acting analogues found difference of HbA1C of only -0.1% (statistically insignificant in both studies) (3,4). The results of this meta-analysis implies that in adults with type 1 diabetes mellitus, CSII shows favourable effects on glycaemic control (greater decrease in HbA1C levels), in participants who had higher HbA1C levels at enrolment than who had closer to the target HbA1C level at enrolment. The insulin pump therapy using rapid acting analogues in comparison to MDI (rapid acting analogues + long acting analogue) had clinically insignificant reduction in HbA1C (1).

Daily mean blood glucose

In 10 studies that included participants more than 18 years of age the mean difference of daily mean blood glucose was estimated to be -18 mg/dl (95% CI -27 to -9) in favour of CSII compared with MDI (5).

Hypoglycaemic events

There was a similar incidence rate of severe hypoglycaemia in the 2 intervention groups (CSII and MDI). The incidence of mild hypoglycaemia and nocturnal hypoglycaemia was also similar (1). This meta-analysis compared CSII using insulin analogues with MDI in which the pre-prandial insulin was also insulin analogue (1).

Quality of life

A meta-analysis of 2 studies favoured CSII over MDI for diabetes mellitus specific QOL (1). There was also improvement in general QOL between the 2 intervention groups favouring CSII.

Weight gain

Weight gain did not differ between CSII and MDI on meta-analysis of 4 studies (1).

Adverse events

Overall, information on adverse treatment effects other than hypoglycaemia is insufficient (6). As per analysis of quality RCTs in Cochrane Review, none of the studies reported on mortality, morbidity or costs (5).

Guidelines recommendations

In the United Kingdom, the National Institute for Health and Clinical Excellence (NICE) has recommended CSII as a cost-effective treatment option in adults with T1DM when attempts to achieve target HbA1c levels with MDI have resulted in disabling hypoglycaemia or when HbA1c levels have remained ≥8.5% despite best efforts (7). As per American Association of Clinical Endocrinologists (AACE) insulin pump management task force, patients with type 1 DM who do not reach glycaemic goals despite adherence to a maximum MDI, are candidates for CSII (8), especially if they have:

i. Very labile DM (erratic and wide glycaemic excursions, including recurrent DKA.
   ii. Frequent severe hypoglycaemia and/or hypo-glycaemia unawareness.
   iii. Significant “dawn phenomenon”, extreme insulin sensitivity.

The information on various outcomes by using CSII, as compared to MDI in T1DM (adults) is summarized in Table 1.

Type 1 DM (Children)

HbA1c

Meta-analysis on 7 RCTs (16 or more weeks of follow-up), compared MDI with CSII in children and adolescents with type 1 diabetes mellitus (1). No difference was found between groups in HbA1c level as compared to baseline. Results were similar among adolescents older than 12 years (combined mean between group difference in change from baseline in HbA1c, -0.10% [95% CI, -0.48% to 0.27%]; and less precise among children aged 12 years or younger (combined mean between-group difference in change from baseline in HbA1c, -0.05% [CI, -1.01% to 0.96%]. The meta-analysis concluded that CSII and MDI have similar effects on HbA1c in children and adolescents with type 1 diabetes mellitus.

Daily mean blood glucose

In 4 studies that included participants less than 18 years of age the mean difference of daily mean blood glucose was estimated to be -4 mg/dl (95% CI -14 to 7) in favour of CSII compared with MDI. The difference was not statistically significant (5).

Hypoglycaemic events

Similar rates of severe hypoglycaemia were found in the 2 intervention groups (CSII and MDI) (1). The risk reduction favoured CSII, although the comparison was not statistically significant. Results were similar in meta-analysis of 3 RCTs in adolescents and of 2 RCTs in children aged 12 years or younger. CSII and MDI had similar effects on nocturnal hypoglycaemia. The meta-analysis concluded that CSII and MDI have similar effects on the incidence of severe hypoglycaemia in children and adolescents with type 1 diabetes mellitus.
Clinical update

A pooled analysis of 2 studies showed no significant difference in general QOL questionnaire (1). A meta-analysis that examined the metabolic and psychosocial impact of CSII and included five paediatric studies reported no consistent differences in anxiety, depression, QOL, self-esteem, and family functioning (9). In qualitative studies using standardized interview techniques, on switching from MDI to CSII, parents of infants and toddlers reported more freedom, flexibility, and spontaneity in their lives as well as reduced parental stress and worry regarding their child’s overall care (10).

Weight gain

A pooled analysis of 2 studies shows no between-group mean difference in weight (1).

Adverse events

None of the studies reported on mortality, morbidity or costs in studies analysed in Cochrane review (5). Individuals using CSII are potentially at increased risk of developing diabetic ketoacidosis (DKA), with DKA rates varying from 2.7 to 9 episodes per 100 patient-years (11). However, as with MDI, DKA is preventable in CSII using published DKA prevention guidelines (12). In Norwegian children with diabetes, the nationwide incidence of DKA (approximately 4 episodes per 100 patient-years) did not change despite an increase in CSII use from 5% in 2001 to 38% in 2005 (13).

Guidelines recommendations
• National Institute for Health and Clinical Excellence (NICE) has recommended CSII as a cost-effective treatment option in adults with T1DM when attempts to achieve target HbA1C levels with MDI have resulted in disabling hypoglycaemia or when HbA1C levels have remained ≥ 8.5% despite best efforts.
• As per American Association of Clinical Endocrinologists insulin pump management task force patients with type 1 DM who do not reach glycaemic goals despite adherence to a maximum MDI, are candidates for CSII especially if they have:
  i. Very labile DM (erratic and wide glycaemic excursions, including recurrent DKA
  ii. Frequent severe hypoglycaemia and/or hypoglycaemia unawareness
  iii. Significant “dawn phenomenon”, extreme insulin sensitivity

Table 1. Effect on outcomes with CSII in comparison to MDI in adults with T1DM

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Which is better? CSII or MDI</th>
</tr>
</thead>
</table>
| HbA1c                         | CSII group had more reduction in HbA1C (Clinically insignificant).
|                               | CSII showed greater decrease in HbA1C levels in participants who had higher HbA1C levels at enrolment. |
| Daily mean blood glucose      | The mean difference of daily mean blood glucose was estimated to be -18 mg/dl in favour of CSII compared with MDI. |
| Hypoglycaemic events          | No difference |
| Quality of life               | There is improvement in general quality of life with CSII. |
| Weight gain                   | No difference |
| Adverse events                | Insufficient information |

Guidelines recommendations
• As per NICE guidelines (7), CSII is recommended as a treatment option when MDI is considered impractical or inappropriate in children <12 years with T1DM, and with the expectation that children would normally undergo a trial of MDI between the ages of 12 and 18 years. CSII should be discontinued (in adults and children ≥12 years who have been started on CSII because of elevated HbA1C or disabling hypoglycaemia) if no sustained improvement
in HbA1c or rate of hypoglycaemic episodes occurs. In children ≥12 years with T1DM, CSII is recommended as a treatment option when attempts to achieve target HbA1c levels with MDI have resulted in disabling hypoglycaemia or when HbA1c levels have remained high (≥8.5%) despite a high level of care (7).

As per joint consensus statement from the European Society for Paediatric Endocrinology (ESPE), The Lawson Wilkins Paediatric Endocrine Society (LWPES), and the International Society for Paediatric and Adolescent Diabetes (ISPAE) (endorsed by the American Diabetes Association and the European Association for the Study of Diabetes) (12), all paediatric patients with type 1 diabetes are potential candidates for CSII, and there is no lower age limit for initiating CSII. CSII should be considered in the conditions listed below:

1. Recurrent severe hypoglycaemia
2. Wide fluctuations in blood glucose levels regardless of A1C
3. Suboptimal diabetes control (i.e., A1C exceeds target range for age)
4. Microvascular complications and/or risk factors for macrovascular complications
5. Good metabolic control but insulin regimen that compromises lifestyle

The information on various outcomes by using CSII, as compared to MDI in T1DM (children and adolescents) is summarized in Table 1.

**Type 2 DM (adults)**

**HbA1c**

A meta-analysis on 4 RCTs, compared CSII with MDI in adults with type 2 diabetes mellitus. CSII used rapid-acting analogue. MDI was based on long-acting analogues with rapid-acting analogues or NPH with rapid-acting analogue/regular insulin in the MDI group (1). This meta-analysis found that CSII decreased HbA1c levels more than MDI did, (combined mean between-group difference was -0.18% [CI, -0.43% to 0.08%] ). The results were not statistically significant, neither have they indicated any clinical advantage of CSII over MDI.

**Hypoglycaemic events**

Analysis of findings of two RCTS found a similar rate of severe hypoglycaemia in the 2 intervention groups (CSII and MDI). The incidence of mild hypoglycaemia was also similar (1).

**Quality of life**

No difference in general QOL or diabetes mellitus-specific QOL between the CSII and MDI intervention groups has been reported (1).

**Weight gain**

Weight gain did not differ between CSII and MDI groups in a meta-analysis of 2 studies (1).

**Guidelines recommendation**

As per NICE guidance, CSII is not generally recommended in type 2 diabetes mellitus, although some subgroups may benefit (7). The AACE recommends CSII in selected patients with insulin requiring type 2 DM who satisfy any or all of the following (8):

1. C-peptide positive but with suboptimal control on a maximal program of basal/bolus injections
2. Substantial “dawn phenomenon”
3. Erratic lifestyle (eg, frequent long distance travel, shift-work, unpredictable schedules leading to difficulty maintaining timing of meals)
4. Severe insulin resistance, candidate for U500 insulin by CSII
5. Selected patients with other DM types (eg, post pancreatectomy)

The information on various outcomes by using CSII, as compared to MDI in T2DM is summarized in Table 3.

**Pregnancy**

The number of randomized controlled trials comparing CSII and MDI in pregnancy is scarce and the available studies have included a small number of participants (14). A majority of the studies conducted in pregnant women are observational in nature involving both type 1 and type 2 diabetics (15). Most of the results are from trials in the 1980s and early 90s when pumps were less reliable and less technically sophisticated (16). These used regular insulin, instead of newer insulin analogues that are available now and are the standard of care. Therefore, the comparison between the CSII and MDI, presented below has to be interpreted keeping these caveats in mind.

**HbA1c**

In Cochrane review, only one RCT fulfilled the eligibility criteria. In this RCT, HbA1c was compared in 16 patients in each arm and in each trimester. There was no statistically significant difference in mean HbA1c in the two arms in any of the trimester. The mean difference in HbA1c was 0.2% in first trimester favouring CSII and was 0.7% and 0.1% favouring MDI in second and third trimester respectively (14). In another meta-analysis, the three studies were included (selection criteria for trials in this meta-analysis were not stringent). The meta-analysis performed on glycosylated hemoglobin at term in 3 studies showed no significant differences between the 2 treatment groups at any period of time (15).
Table 2. Effect on outcomes with CSII in comparison to MDI in children and adolescents with T1DM

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Which is better? CSII or MDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>No difference</td>
</tr>
<tr>
<td>Daily mean blood glucose</td>
<td>No difference</td>
</tr>
<tr>
<td>Hypoglycaemic events</td>
<td>No difference</td>
</tr>
<tr>
<td>Quality of life</td>
<td>No difference in general QOL score</td>
</tr>
<tr>
<td>Weight gain</td>
<td>No difference</td>
</tr>
<tr>
<td>Adverse events</td>
<td>No difference (DKA episodes)</td>
</tr>
<tr>
<td>Guidelines recommendations</td>
<td>NICE guidance</td>
</tr>
<tr>
<td></td>
<td>• CSII is recommended as a treatment option when MDI is considered impractical or inappropriate in children &lt;12 years with T1DM</td>
</tr>
<tr>
<td></td>
<td>• CSII is recommended as a treatment option when attempts to achieve target HbA1c levels with MDI have resulted in disabling hypoglycaemia or when HbA1c levels have remained high (≥8.5%) despite a high level of care.</td>
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<td>ISPAD, LWPES, ESPE recommendations</td>
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<td></td>
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</tr>
</tbody>
</table>

Table 3. Effect on outcomes with CSII in comparison to MDI in T2DM

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Which is better? CSII or MDI</th>
</tr>
</thead>
<tbody>
<tr>
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<td>No difference</td>
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<tr>
<td></td>
<td>2. Substantial “dawn phenomenon”</td>
</tr>
<tr>
<td></td>
<td>3. Erratic lifestyle (eg, frequent long distance travel, shift-work, unpredictable schedules leading to difficulty maintaining timing of meals)</td>
</tr>
<tr>
<td></td>
<td>4. Severe insulin resistance, candidate for U500 insulin by CSII. Selected patients with other DM types (eg, postpancreatectomy)</td>
</tr>
</tbody>
</table>
Continuous subcutaneous insulin infusion versus multiple dose insulin

Maternal 24 hour mean blood glucose (mg/dl)

The meta-analysis of 3 studies revealed insignificant (0.12 mg/dl) difference in maternal 24 hour mean blood glucose in first trimester between the two arms. The difference was 1.77 mg/dl and 0.08 mg/dl in second and third trimester respectively, all favouring MDI (14).

Maternal hypoglycaemia

In a meta-analysis by Mukhopadhay et al, relative to women allocated to MDI therapy, women who received CSII had more hypoglycaemic spells (22.3% vs 19.2%; OR, 1.34, p=0.46). This meta-analysis was reported on 5 studies (15). In another meta-analysis, the risk ratio was 3.0 with more hypoglycaemic events in CSII than in MDI. The number of hypoglycaemic events was only 3 in 30 total patients on CSII as compared to in 1 patient out of 31 patients in MDI group (14).

Neonatal hypoglycaemia

Neonatal hypoglycaemia was reported from 5 studies in a meta-analysis. There was no significant difference between the two treatment groups (19.1% in CSII vs 14.8% in MDI group; OR, 1.31, P=0.51) (15).

Ketoacidosis

The meta-analysis of 4 studies, (published from 1986 to 1993), have shown more episodes of ketoacidosis in CSII group (5 episodes in pooled 70 subjects in CSII group as compared to none in 73 subjects in MDI group, p=0.23) (15).

Caesarean rates

Caesarean section rates did not differ between the CSII and MDI groups (51% vs 43%; OR, 1.39, p=0.27) in a meta-analysis of 5 studies (15).

Perinatal mortality

There were 6 stillbirths in CSII group (n=94) and 1 in MDI group (n=88) in pooled analysis of 5 studies. The OR being 2.5, but was not statistically significant (p=0.25) (15).

Table 4. Effect on outcomes with CSII in comparison to MDI in pregnancy

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Which is better? CSII or MDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
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</tr>
<tr>
<td>Maternal 24 hour mean blood glucose (mg/dl)</td>
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</tr>
<tr>
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<td>Relatively more with CSII</td>
</tr>
<tr>
<td>Neonatal hypoglycaemia</td>
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</tr>
<tr>
<td>Ketoacidosis</td>
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</tr>
<tr>
<td>Caesarean rates</td>
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</tr>
<tr>
<td>Perinatal mortality</td>
<td>Relatively more with CSII</td>
</tr>
<tr>
<td>Neonatal birth weight, small or large for gestational age</td>
<td>No significant difference</td>
</tr>
</tbody>
</table>

Guidelines opinion

- As per NICE guidance, CSII should be considered in pregnancy or pre-conceptually in women with T1DM when the target HbA1c (normally ≤6.1%) in the first trimester or pre-conceptually cannot be achieved without disabling hypoglycaemia.
- As per AACE, the literature does not suggest clear evidence that insulin pumps are necessary for optimal treatment of women with type 1 DM during pregnancy. A robust randomized trial, adequately powered to assess efficacy outcomes for CSII vs MDI in pregnant women with DM, is needed.
- AACE opines that insulin pump therapy seems to be safe and effective for maintaining glycaemic control in pregnancies complicated by gestational DM/type 2 DM requiring large doses of insulin.
Neonatal birth weight, small or large for gestational age

There was no significant difference in the birth weight, rates for small for gestational age or large for gestational age between the two treatment groups (15).

Guidelines recommendations

As per NICE guidance, CSII should be considered in pregnancy or pre-conceptually in women with T1DM when the target HbA1c (normally ≤ 6.1%) in the first trimester or pre-conceptually cannot be achieved without disabling hypoglycaemia (7). As per AACE, the literature does not suggest clear evidence that insulin pumps are necessary for optimal treatment of women with type 1 DM during pregnancy. A robust randomized trial, adequately powered to assess efficacy outcomes for CSII vs MDI in pregnant women with DM, is needed. On the basis of one study, AACE opines that insulin pump therapy seems to be safe and effective for maintaining glycaemic control in pregnancies complicated by gestational DM/type 2 DM requiring large doses of insulin (17).

The information on various outcomes by using CSII, as compared to MDI in pregnancy is summarized in Table 4.

Apart from the specific points that were discussed under different groups of diabetes, there are some other important considerations. Glycaemic variability is a major frustration for patients with T1DM. High glycaemic variability correlates with an increased frequency of hypoglycaemia and individuals with the most variability maintain the highest HbA1c level on MDI, probably to avoid increasing the frequency of hypoglycaemia, CSII reduces both the within-day and day-to-day variability (16). This improvement is probably because the large variation in subcutaneous absorption associated with large injected volumes of long-acting insulin (±50% for isophane) is reduced to about ±3% with CSII (probably because there is a subcutaneous insulin depot of only about 1 unit at any time during basal rate infusion) (18). The frequency of diabetic ketoacidosis is not significantly different during modern CSII versus MDI; however, the potential risk of ketoacidosis is greater with CSII in the event of pump malfunction with interrupted insulin delivery, or with the increased insulin requirements of illness, because of the smaller subcutaneous insulin depot with CSII.

The selection of an optimal candidate for this complex therapy is important. Clearly, CSII is not appropriate for every patient with insulin-requiring DM. The ideal CSII candidate would be a patient with type 1 DM or absolutely insulin-deficient type 2 DM who currently performs 4 or more insulin injections and 4 or more self-monitored blood glucose measurements daily, is motivated to achieve tighter blood glucose control, is willing, and intellectually and physically able to undergo the rigors of insulin pump therapy initiation and maintenance. Eligible patients should be capable of self-management through frequent self-monitored blood glucose measurements. Further, candidates must be able to master carbohydrate counting and insulin correction and adjustment formulas and must be prepared to troubleshoot problems related to pump operation and blood glucose levels. Last, patients should be emotionally mature, with a stable life situation, and willing to maintain frequent contact with members of their health care team, in particular their pump-supervising physician. Monomeric, rapid-acting insulin analogues (aspart, lispro, or glulisine) are now considered to be the insulin of choice for pumps (19). Insulin aspart is approved for use in children 2 years and above. Lispro and Glulisine are approved in children above 3 and 4 years respectively. Insulin aspart and lispro are approved for use in pregnancy.

Conclusions

MDI regimen that includes frequent self-monitoring of blood glucose levels and structured diabetes education can achieve good glycaemic control in many individuals with T1DM. A trial of CSII is indicated in patients who do not achieve acceptable glycaemic control with MDI because of continued, elevated HbA1c levels or disabling hypoglycaemic episodes. Continuous subcutaneous insulin infusion (CSII) can reduce HbA1c levels and hypoglycaemia in many adult patients with type 1 diabetes mellitus (T1DM), compared with multiple daily insulin injections (MDI). The greatest reduction in HbA1c levels with CSII occurs in patients with T1DM who have the worst glycaemic control with MDI. Because of the curvilinear relationship between HbA1c level and microvascular risk, reductions in HbA1c level from this high starting point produce a more marked reduction in the risk of diabetic complications and better cost-effectiveness than that achievable for unselected patients with a lower mean HbA1c level. NICE in the UK judged that, when quality of life improvements are taken into account along with reductions in HbA1c level, CSII is cost-effective when HbA1c levels on MDI are ≥8.5% and, therefore, used this cut-off value in their guidance. Blood glucose variability and treatment satisfaction are also usually improved with CSII versus MDI. CSII is not generally recommended in type 2 diabetes mellitus, although some subgroups may benefit. CSII is safe and effective in pregnancy, but no convincing evidence exists from either observational studies or the relatively few RCTs conducted that glycaemic control or pregnancy outcomes differ between CSII and MDI.
References


An adrenal tumour secreting multiple hormones
H N Rajaratnam1, S A Abhayaratna2, N P Somasundaram3, A Samarasekara4, S K Kollure5
Sri Lanka Journal of Diabetes, Endocrinology and Metabolism 2014; 4: 30-34

Case report

A right homogeneous adrenal tumor was found incidentally, during abdominal computed tomography in a 72-year-old female patient, presenting with hypertension of 2 years duration. She had an elevated Aldosterone: Renin Ratio (ARR) and primary hyperaldosteronism was confirmed with a fludrocortisone suppression test. Plasma basal cortisol and adrenocorticotropic hormone (ACTH) levels were normal, but the plasma cortisol concentration could not be suppressed with dexamethasone. Therefore, an adrenal cortical adenoma with primary hyperaldosteronism and subclinical hypercortisolism was suspected. Urinary total metanephrines and vanillylmandelic acid (VMA) levels were also marginally elevated, indicating the possibility of a pheochromocytoma. After right adrenalectomy, the tumour was histologically demonstrated to be a pheochromocytoma, and the levels of all three hormone groups viz corticosteroid, mineralocorticoid and metanephrine levels normalized, indicating the possibility of a co-secretory small adrenocortical microadenoma.

Key words: cortical adenoma, pheochromocytoma, subclinical hypercortisolism

Introduction
Before the era of computed tomography (CT) and magnetic resonance imaging (MRI), adrenal tumours were identified by the detection of excessive hormone levels. Presently, due to technological advances and wide availability of imaging modalities, these tumours are often discovered incidentally during CT scanning or ultrasonography undertaken for other reasons and are called “incidentalomas”. In most of the cases, clinical and biochemical features of excessive hormone levels are absent, but some of them demonstrate normal basal hormone levels with abnormal responses to dynamic testing. The prevalence of adrenal incidentalomas had been shown to be as high as 10% in older patients (1).

We describe a case of an elderly woman with hypertension, who was incidentally discovered to have an adrenal tumour suggestive of a cortical adenoma, which was later proved to be a pheochromocytoma.

Case report
A 72-year-old woman presented with a three month history of episodic vomiting and intermittent pain in the right upper quadrant of the abdomen. Her routine ultrasonography detected a 3-cm-wide mass in the right adrenal gland. CT scanning confirmed the finding and revealed a well-defined homogenous, hypodense mass lesion, measuring 3.5 × 3.0 cm, in the right supra renal region, with marked enhancement after contrast. There was no hyper or hypoplasia in the contralateral left adrenal gland (Figure 1).

In the past, the patient had suffered from mild hypertension, hypothyroidism, and osteopaenia of the lumbar spine and hip (spine T score -1.7, Z -0.6, hip T -2.0, Z -0.7) of 2 , 10 and 2 years respectively. For these, she was treated with long acting calcium channel blockers, levothyroxine and raloxifene. Recently, she had experienced occasional palpitations without any episodic headache or sweating, and her blood pressure control deteriorated despite good compliance with treatment.

Her BMI was 18.3 kg/m². There were no classical signs of Cushing syndrome such as purple striae, facial plethora, dorsocervical fat pad or ecchymosis. Blood pressure was 160/ 90 mmHg, and pulse rate was 90/min. No enlarged thyroid was palpable. There were no other abnormal clinical signs.

Her baseline investigations including serum calcium, creatinine and electrolyte levels were normal. In view of incidentally detected adrenal mass, further adrenal hormone evaluation was undertaken. (normal values are

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An adrenal tumour secreting multiple hormones

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The morning serum cortisol level was 27 mcg/dL (4 to 30 mcg/dL). Following administration of dexamethasone, 0.5 mg every 6 hours for 48 hours, and thereafter 2 mg every 6 hours for 48 hours, the serum cortisol levels were 4.47 (1.8 mcg/dL) and 2.8 mcg/dL (>50% reduction) respectively. The failure of dexamethasone to suppress cortisol production in the above tests, indicated autonomous cortisol production from the adrenal gland.

The serum levels of ACTH was 15 pg/mL (0 to 46 pg/mL), testosterone 0.3ng/mL (0.0 to 0.7ng/mL), T4 1.23 ng/dL (0.89-1.76 ng/dL) and TSH 2.3 IU/mL (0.4-4 IU/mL). The plasma renin activity and aldosterone level at 8 am, after being ambulatory for 2 hours and seated for 15 minutes were 0.68 ng/mL/hr (1-4 ng/mL/hr) and 21.87 ng/dL (5-30 ng/dL) respectively. The ARR was high at 32 (normal < 20) which was suggestive of primary aldosteronism. The 24-hour urinary VMA level of 18.7 mg/24 hours (1 to 11 mg/24 hours) and the 24 hour urinary metanephrines of 410 ng/mL (0-350 ng/mL) were suggestive of increased catecholamine secretion. Fludrocortisone suppression test with fludrocortisone 0.1mg 6 hourly orally for 4 days with salt loading and close monitoring of the serum potassium and blood pressure levels was performed, to confirm the diagnosis of primary hyperaldosteronism. Pre and post test aldosterone samples were measured (Table 1), which indicated excess aldosterone secretion. The next logical procedure was to localize the source of aldosterone by adrenal venous sampling. This was not performed because of the cost, and lack of access to the procedure. The patient underwent surgical removal of the right adrenal gland (Table 1).

![Figure 1. a) Pre-contrast CT scan showing the right sided well defined hypodense adrenal tumour (white arrow). b) Post-contrast CT image showing the contrast enhancement (black arrow).](image)

<table>
<thead>
<tr>
<th>Table 1. Fludrocortisone suppression test</th>
</tr>
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<tbody>
<tr>
<td>Basal plasma aldosterone – upright</td>
</tr>
<tr>
<td>Plasma aldosterone on day 4 of fludrocortisone – upright</td>
</tr>
</tbody>
</table>
Though a right adrenocortical adenoma was suspected based on conspicuous biochemical and imaging characteristics, marginally elevated urinary VMA and metanephrines mandated a consideration of a functioning pheochromocytoma (primary or coexisting) in this patient. The patient was given oral phenoxybenzamine for 10 days preoperatively, starting with 10 mg 12 hourly and gradually increasing up to 20 mg 12 hourly. With treatment, the blood pressure came down to normal levels (systolic BP 110 to 80 mmHg and diastolic BP 80 to 60 mmHg). The patient was also given propanolol 10 mg three times daily for 5 days, starting from day eleven. She underwent laparoscopic right adrenalectomy. Intra operative and post-operative periods were uneventful. Hydrocortisone replacement therapy was started to counter possible post-operative hypocortisolism, caused by suppression of the contralateral adrenal gland by the hypercortisolism produced by the autonomous adrenal tumour. She was discharged on post-operative day 5 on a single antihypertensive agent.

Figure 2. A) H & E section shows tumor cells B) Chromogranin A immunostaining is diffusely positive in the tumor cells. C) Tumour cells negative for cytokeratin (CK) immunostaining. D) Synaptophysin staining shows diffuse cytoplasmic staining in the tumor cells.
One month after surgery, hydrocortisone was omitted after recording normal baseline values without replacement. The blood pressure was well controlled on prazosin 0.5 mg t.d.s. Post-operative hormonal analysis revealed a fasting basal serum cortisol level of 12.5 mcg/dL, suppressed to less than 1.8 mcg/dL with the low dose dexamethasone suppression test, ARR of 16.5 (<20) and a 24 hour urine metanephrines of 38.6 ng/mL (0-350 ng/mL). Histopathology of the right adrenal gland revealed the tumour to be a pheochromocytoma with surrounding normal adrenal tissue. Immunohistochemical staining of the tumour demonstrated cells that were strongly positive for chromogranin A, synaptophysin and S100, while it was negative for cytokeratin (CK). Surrounding adrenal tissue showed normal adrenal gland features and failed to show any other type of tumour (Figure 2).

**Discussion**

Pheochromocytomas and adrenocortical adenomas derive from distinct origins. Therefore, simultaneous occurrence of these two entities is extremely rare, especially in the same adrenal gland. There had only been a few cases of pheochromocytomas, either with functioning or non-functioning adrenal cortical adenoma, reported in the literature (2). At diagnosis, the patient's tumour was thought to be non-functional, because she didn't have definitive signs or symptoms of a syndrome associated with excessive levels of adrenal hormones, except for hypertension. However autonomous secretion of aldosterone, cortisol and catecholamines was established after hormonal evaluation.

Subclinical Cushing syndrome (i.e., glucocorticoid secretory autonomy without clinical manifestations of Cushing syndrome) is the most frequent hormonal abnormality detected in patients with adrenal incidentalomas (3). Because of the lack of sensitivity of most ACTH assays at the lower part of the reference range, most centers rely on an alternative measure of autonomous cortisol secretion such as the overnight dexamethasone suppression test (DST). Hyper functioning of both the medulla and cortex of an adrenal gland is more commonly seen when a pheochromocytoma secretes ACTH or corticotrophin releasing factor (CRF) ectopically. This was not the case in our patient, as she had a low normal level of ACTH and had no hyperplasia in the contra lateral adrenal gland. Non suppressed cortisol following 8 mg/d of dexamethasone for 2 days, suggested that the excess cortisol was of adrenal origin. The simultaneous hyper secretion of catecholamines, cortisol and aldosterone can be explained by many mechanisms. Catecholamines have been shown to have an effect on steroidogenesis of adjacent cortical cells in a paracrine manner (4). Human pheochromocytomas may also synthesize and secrete several regulatory peptides, such as adrenomedullin, somatostatin, neuropeptide Y, and galanin, which are able to influence adrenocortical steroid production. Long term secretion of ACTH, catecholamines and several other peptides might result in adrenal cortical adenoma formation (4). However, it is still difficult to explain why most patients with pheochromocytoma do not develop cortical hyperplasia or adenomas.

Pheochromocytomas are being increasingly discovered in the presymptomatic stage, due to the widespread use of CT and MRI. Approximately 3 percent of adrenal incidentalomas prove to be pheochromocytomas (5). Although in pheochromocytomas, urine metanephrines are elevated 2-3 times the upper limit of normal value, a study revealed that thirty percent of patients with adrenal incidentalomas have a final diagnosis of pheochromocytoma with urine or plasma metanephrines which were only borderline elevated (6).

Adrenocortical carcinomas have been reported to secrete multiple hormones including catecholamines (7). They are usually large at diagnosis (more than 4 cm) and appear heterogeneous in images owing to the presence of internal hemorrhage and necrosis (8). These features were not seen in this patient. They have distinct histological features and immunohistochemical staining which help to differentiate them from a pheochromocytoma. Both pheochromocytoma and adrenal cortical carcinoma are immunoreactive for synaptophysin, although the staining with a pheochromocytoma is much stronger. Chromogranin A, another diffuse neuroendocrine marker, is not positive in adrenal cortical tissues, making it one of the most useful immunohistochemical markers in distinguishing adrenal medullary tumors from adrenal cortical tumors. Cytokeratins, which are positive in adrenal cortical tumors, are also helpful in distinguishing between these two entities (9). In this patient there was no evidence to suggest the presence of an adrenocortical carcinoma and immunohistochemical staining of the tumour confirmed that it was of medullary origin.

This patient had unequivocal evidence of autonomous aldosterone secretion which resolved after the tumour removal. Although the “imaging phenotype” of the tumour in the right adrenal gland (less than 4 cm, homogenous, hypodense round mass with regular borders) was more in favour of a cortical adenoma, histology and immunohistochemical staining proved it to be a pheochromocytoma. Unfortunately, we couldn’t locate the exact source of the excess aldosterone. The most possible explanation for this would be that even though careful histological analysis of the specimens was done, there may have been a small aldosterone producing cortical microadenoma, which may not have been included in the cut up specimens taken up for histology. The fact
that patient’s ARR normalized after surgery, made this the most likely explanation, than a missed small aldosterone producing adenoma in the contralateral adrenal gland. The current guidelines recommend performing adrenal venous sampling to localize the site of aldosterone producing tumour, before proceeding to surgery (10). Since adrenal incidentalomas are common, this is a safe approach in those above 40 years of age.

About 10 percent of all catecholamine-secreting tumors are malignant (11). Malignant pheochromocytomas are histologically and biochemically similar to benign ones and the only true indicator of malignant behaviour is metastatic spread. Our patient was advised to attend the follow up clinic annually, with hormonal assessment to detect any recurrence.

This case is a good example to emphasize the fact that careful and detailed endocrine evaluation is warranted in each patient with an incidentally discovered adrenal mass. With the available resources, we were able to reasonably establish that the patient’s right adrenal gland had autonomous mixed hormonal secretion.

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Idiopathic central precocious puberty associated with an enlarged pituitary gland

S Pathmanathan¹, Navoda Atapattu², N P Somasundaram³, K S H de Silva⁴


Abstract

Precocious puberty is a rare condition characterized by the development of secondary sexual characteristics before the median age for the sex. We report a 5 year and 7 month old girl who presented to us with precocious puberty who had a large anterior pituitary for the age.

Key words: central precocious puberty (CPP), magnetic resonance imaging (MRI), pituitary gland

Introduction

Precocious puberty in girls is defined as the development of secondary sexual characteristics before the age of eight years. There are two types, gonadotrophin dependent, or gonadotrophin independent. Gonadotrophin dependent precocious puberty is due to the premature activation of the hypothalamo-pituitary-ovarian axis, also known as central precocious puberty (CPP) (1). CPP in girls is idiopathic in the majority of cases (2).

Magnetic resonance imaging (MRI) of the head with special attention to the hypothalamo-pituitary region is indicated in patients with CPP to look for a tumor or an intracerebral lesion after hormonal studies indicate a diagnosis of central precocious puberty (3, 4). The size and shape of the normal pituitary gland vary considerably which is also affected by age, sex, and hormonal environment (5, 6, 7). The increase in pituitary height and volume during puberty is well known (8, 9). This increase during puberty is related to the changes in the endocrine hormone secretion during puberty specially due to the hyper-secretion of luteinizing hormone (LH) during this period (10, 11). These morphological changes can mimic pituitary adenomas causing confusion in the diagnostic process. Therefore careful evaluation by a multidisciplinary team and correct interpretation of the imaging is important in the management of CPP before appropriate treatment is instituted.

We describe a child with central precocious puberty whose MRI of the brain was suggestive of an anterior pituitary tumour without mass effect. After careful evaluation and interpretation it was concluded that the increase in size in the pituitary gland is a normal variation due to the premature activation of the hypothalamo-pituitary-ovarian axes.

Case presentation

A 5 year and 7 month old baby girl was referred to our pediatric endocrinology unit for evaluation of bilateral breast enlargement and growth of pubic hair of six months duration followed by one episode of vaginal spotting, initially noticed one week prior to her presentation. Her birth history was unremarkable and her growth and development were normal until 3 years of age. From 3 years of age her parents noticed she was growing taller than her elder sibling who was 1 year and 5 months older than her. She was noticed to be crossing her centiles in both height and weight at well baby clinics (Figure 1). There was no history of early morning headache, vomiting, visual disturbances, fits or past history of cranial irradiation. There was no family history of endocrinological disorders or precocious puberty. At presentation, our patient was an alert child. Her height was 132 cm (>97th percentile) and 34.8 kg in weight (>97th percentile). Her vital signs were normal. She had normal ocular, thyroid and cardiorespiratory examinations. She had Tanner stage III breast development and pubic hair of Tanner stage II.

Her bone age was 11 years at a chronological age of 5 years and 7 months. USS abdomen revealed normal ovaries but the uterus was large for age (5.8 × 2.7 × 2.2 cm)
and adrenals were normal. Her haematological and biochemical parameters were within normal limits. Hormonal assay revealed the following: 8 am serum follicle stimulating hormone (FSH) and LH were 8.24 mIU/ml (follicular phase - 2.8 -11.3) and 2.08 mIU/ml (follicular phase - 1.1 -11.6) respectively while midnight FSH and LH were 3.85 mIU/ml (follicular phase - 2.8 -11.3) and 3.77 mIU/ml (follicular phase - 1.1 -11.6) respectively. Serum estradiol 59 pg/ml (39 -189), 3rd gen TSH - 2.29 mIU/ml (6y - 0.34-6.0), 8 am serum cortisol - 13.8 μg/dl (2-6 years 1-26), serum prolactin - 8.32 ng/ml (6-10 years -1.2-11.4) and serum Insulin like Growth Factor (IGF 1) – 628 ng/ml (6yr - 52-297) (11 yr - 111 -551). Hormonal assay revealed elevated levels of serum FSH, LH, and serum estradiol confirming the clinical diagnosis of CPP. Interestingly her serum IGF-1 was elevated even when compared to her advanced bone age.

Magnetic resonance imaging (MRI) of the brain was reported to have an early anterior pituitary tumour without mass effect (Figure 2 & 3). Considering the elevated serum IGF level and the MRI findings the patient underwent a standard 75mg oral glucose tolerance test (OGTT)(Table 1).

**Figure 1. CHDR of the patient.**
Idiopathic central precocious puberty associated with an enlarged pituitary gland

Figure 2. MRI scan of brain – (pre contrast).

Figure 3. MRI scan of brain – (post contrast).

Table 1. Results of glucose suppressed growth hormone (GH) (75 g OGTT)

<table>
<thead>
<tr>
<th>Time</th>
<th>Serum GH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>1.47 ng/ml</td>
</tr>
<tr>
<td>½ hr</td>
<td>0.79 ng/ml</td>
</tr>
<tr>
<td>1 hr</td>
<td>2.5 ng/ml</td>
</tr>
<tr>
<td>1 ½ hr</td>
<td>3.15 ng/ml</td>
</tr>
<tr>
<td>2 hr</td>
<td>2.42 ng/ml</td>
</tr>
</tbody>
</table>
As the 75g OGTT was inconclusive she also underwent a 9 point growth hormone (GH) assessment, which showed a normal pulsatile secretory pattern of GH (Table 2).

<table>
<thead>
<tr>
<th>Time</th>
<th>GH</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 a.m.</td>
<td>&lt;1.0 μu/L</td>
</tr>
<tr>
<td>10 a.m.</td>
<td>9.2 μu/L</td>
</tr>
<tr>
<td>12 noon</td>
<td>1.0 μu/L</td>
</tr>
<tr>
<td>2 p.m.</td>
<td>&lt;1.0 μu/L</td>
</tr>
<tr>
<td>4 p.m.</td>
<td>1.0 μu/L</td>
</tr>
<tr>
<td>6 p.m.</td>
<td>9.9 μu/L</td>
</tr>
<tr>
<td>8 p.m.</td>
<td>&lt;1.0 μu/L</td>
</tr>
<tr>
<td>10 p.m.</td>
<td>9.3 μu/L</td>
</tr>
<tr>
<td>12 midnight</td>
<td>18.4 μu/L</td>
</tr>
</tbody>
</table>

It has been reported that the size of the pituitary gland enlarges during puberty (10,11,12). This was the reason for the MRI findings and the normal GH assay in our patient. After proper counseling of the parents it was decided to treat the patient with depot Goserelin acetate 3.6 mg monthly. After 9 months of follow up, the child’s growth stabilized with regression of breast tissue and cessation of menstruation.

Discussion

MRI is not indicated in all patients presenting with CPP (3). For healthy girls aged 6-8 years with no signs or symptoms of CNS disease, the likelihood of finding a tumor or intracerebral pathology is only about 2%; therefore, this test may be unnecessary depending on the clinical situation. But among children younger than 6 years there is a greater chance of finding CNS pathology, therefore MRI is routinely indicated in these children (4).

With the recent advances, MRI has become the investigation of choice for visualizing intracranial structures including the pituitary gland. Various studies depending on MRI findings of the pituitary in different age groups, have described the dynamic changes in the size, shape and signal intensity of the pituitary in these age groups, which reflects the changes in the complex hormonal environment of this gland (5,6,7). These changes were more significant in females. The height of the anterior-pituitary gland increases between birth and 15 years, with two peaks, one during the first year of life and the second during puberty. Increased secretion of LH and FSH is the main cause of increased volume of the pituitary gland at puberty. It remains unchanged until the age of 40 years, after which it decreases (8). This decrease occurs despite of an increase in gonadotrophin secretion at this age. It has also been found that pituitary volume followed the same morphological growth pattern that is reported for pituitary height. The maximum volume of the gland was achieved in the second decade of life for females and third decade for males (9).

There are also published data which have compared MRI findings of the pituitary glands of children with central precocious puberty with age and sex matched control subjects. These studies were helpful in defining MRI-derived variables in the diagnosis of central precocious puberty, and to correlate them with the hormonal profile and other imaging and clinical findings. These variables were pituitary height, length, width, midsagittal cross-sectional area, calculated volume, and shape. The shape was assessed by a pituitary grading system which was defined by the concavity of the upper pituitary surface (grade 1 = marked concavity, grade 2 = mild concavity, grade 3 = flat, grade 4 = mild convexity, grade 5 = marked convexity). The results concluded that pituitary size and shape correlated with the hormonal profile and change in pituitary grade is the most helpful variable for the diagnosis of central precocious puberty in a prepubertal child (10,11,12).

Conclusion

In conclusion, this report emphasizes the need for careful interpretation of the MRI imaging before arriving at a diagnosis in patients with CPP. The normal variation of enlargement in height and volume of the pituitary gland should always be considered in the differential diagnosis of the pituitary enlargement in these patients.

References


Malignant phaeochromocytoma – a challenge in diagnosis and therapy

Dharshini Karuppiah¹, Uditha Bulugahapitiya²

Abstract

Phaeochromocytoma is an endocrine tumour that originates in catecholamine producing chromaffin cells of the adrenal medulla. Approximately 10% are malignant but there are no precise histological or biochemical markers to distinguish these from benign ones. The presence of metastases at distant sites is the most reliable clue but histologic features utilized in several scoring systems aid in predicting malignancy. Malignant phaeochromocytoma predominantly secrete noradrenaline and there may be high dopamine levels. Increased levels of chromogranin A, negative staining for inhibin/activin beta subunit and presence of SDHB mutation are the other factors associated with malignant potential. Multi modality evaluation with combination of CT, MRI, SPECT and radionuclide scintigraphy augments the diagnostic yield. Recent advance in molecular diagnostic markers further improved the knowledge in predicting malignant potential. Currently available therapeutic options are surgical debulking, pharmacological therapy for excess catecholamines, radionuclide therapy, antineoplastic therapy and external radiotherapy. These modalities provide symptomatic relief and biochemical control, but with no significant survival benefit. The development in the field of molecular pathway responsible for the malignant potential of phaeochromocytoma gives a hope to future therapy.

Abbreviations: CT- computed tomography, MRI- magnetic resonance imaging, SPECT- single photon emission computed tomography, PET- positron emission tomography, SDHB- succinate dehydrogenase B, ETA and ETB-endothelin receptors, mTOR- mammalian target of rapamycin, HIF- hypoxia inducible factors, ERBB2- erythroblastic leukemia viral oncogene homolog 2

Key words: malignant phaeochromocytoma, catecholamines, tumour markers

Introduction

Catecholamine secreting tumours are rare neoplasms that arise from chromaffin cells of the adrenal medulla (phaeochromocytoma) and the sympathetic ganglia (paraganglioma). The majority of pheochromocytomas are sporadic, but increasing prevalence of hereditary forms have been demonstrated recently (1). Around 10% of these tumours are malignant (2,3) and despite advanced diagnostic methods prediction of malignancy is difficult. Histologically and biochemically malignant tumours cannot be differentiated from benign ones. The presence of tumour spread to distant sites where chromaffin tissue is normally absent such as lymph nodes, liver, lung and bones is the only reliable clue to the presence of malignancy (4). Standard therapies for malignant phaeochromocytoma are non specific and the rarity of this condition makes it difficult to gather knowledge regarding outcomes of new forms of therapy. Since malignant phaeochromocytoma carries a poor prognosis and the occurrence of metastasis even years after the primary surgery, improvement in diagnostic and therapeutic measures are crucial (5,6).

Diagnostic clues for malignant phaeochromocytoma

Classically the diagnosis of phaeochromocytoma is achieved by measurement of urinary and plasma metanephrines. Methylation of noradrenaline to adrenaline requires phenylethanolamine-N-methyl transferase, which is a cortisol dependent enzyme. This explains the predominant production of adrenaline by phaeochromocytoma and noradrenaline by paraganglioma (7). Malignant phaeochromocytomas secrete predominantly noradrenaline. It is possibly due to the large tumour size which gets direct blood supply rather than corticomedullary. Therefore low cortisol concentration causes lack of methylation to adrenaline (7,8). In addition, increased levels of plasma dopamine or its metabolite methoxytyramine due to less differentiated catecholamine biosynthetic pathway may suggest malignancy (9,10).

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Chromogranin A is a protein released from storage granules of neuroendocrine cells and is elevated in 80% of patients with phaeochromocytoma, though it is not specific, malignancy is usually associated with very high rates (9,11). Some studies show normal adrenal medulla and benign phaeochromocytomas strongly stained for inhibin/activin beta subunit whereas it is negative in malignant tumours which may be used as an indicator of malignant potential (12). The other most important predictor of malignancy is the presence of SDHB mutation (13).

While the imaging features of this entity are variable, the use of coupled multi modality evaluation with CT, MRI, SPECT and radionuclide scintigraphy augment the diagnostic yield. In MRI these tumours have classically been described to have a “light-bulb” hyperintensity on T2-weighted sequences, due to a cystic component, and are hypointense on T1 sequences (14). The signal intensity on T2-weighted images may be low in malignant lesions due to haemorrhagic and necrotic areas though not a sufficient discriminating feature (15). The most specific radiotracer used is $^{123}$I or $^{131}$I labeled metaiodo benzyl guanidine (MIBG), which has chemical similarities to norepinephrine and is concentrated in chromaffin tissue. It permits evaluation of extra adrenal, metastatic and multiple tumours and has the ability to perform whole body scan. But dopamine secreting tumours usually do not take up MIBG and its sensitivity is low in malignant (16). As these chromaffin tumours express somatostatin receptors, Indium-11-DTPA-octreotide, a somatostatin analog can be useful in MIBG negative cases with sensitivity of nearly 90% (17). Detecting small lesions which express low density somatostatin receptors, PET imaging with gallium labeled octreotide ($^{68}$Ga-DOTA TOC) is superior and identification of lung or bone metastases is better with this modality (18). PET imaging with $^{18}$F-FDG has higher sensitivity in detecting glucose avid metastatic lesions (19).

There are no precise histological features to predict malignant potential, but cytologic features, mitotic activity, pattern of growth and invasion are utilized to develop scoring systems. The Phaeochromocytoma of the Adrenal gland Scale Score (PASS) is the most commonly used system and score of more than 6 suggest a malignant lesion (20). In addition the growing development of molecular diagnostic markers provides supplementary information. Studies show Ki 67 index, a marker of proliferation, if $>$4% and absence of pS100 staining have significant correlation with high risk of malignancy and recurrence (21). Up regulation of heat shock protein 90 (HSP90), telomerase activity and the telomerase catalytic subunit (hTERT) are closely linked with malignant phaeochromocytoma (22). It seems overexpression of angiogenic molecules such as vascular endothelial growth factors (VEGF), angiopeptin-2, and endothelin receptors ETA and ETB are also associated with malignant phenotype (23).

Management

No randomized clinical trials are available regarding the treatment of this rare condition. The treatment goals are surgical debulking of primary tumour and metastases if possible, pharmacological control of hormone mediated symptoms, radiometabolic treatment and external radiotherapy, antineoplastic agents and the novel molecular targeted therapy. For large tumours transabdominal approach is preferred over laparoscopic resection and total adrenalectomy with loco regional lymph node clearance is recommended (24). Though surgery alone hardly cures the malignant phaeo-chromocytoma, it reduces the symptoms of catecholamine excess and improves the response to other therapeutic modalities such as MIBG. Arterial chemo-embolization, radiofrequency ablation and stereotactic radiotherapy are the valuable tools for control of metastases (25). Radionuclide treatment with $^{131}$I MIBG is the most widely studied non surgical therapy for tumors which are not resectable and considered as a good option in patients with high uptake on scintigraphy as 60% of metastases are MIBG avid (26,27). Combination with radiolabelled somatostatin analogues has synergistic effect. External radiotherapy might help bone metastases, but the patient needs proper preparation and monitoring to avoid crisis related with excessive release of catecholamine from radio induced inflammation (28). Chemotherapy with combination of cyclophosphamide, vincristine, and dacarbazine (CVD) is the most effective option for lesions which are resistant to both surgery and radionuclide therapy (26). But the tumour has the tendency to recur once the chemotherapy is stopped and becomes unresponsive to the same regimen (29).

Currently available therapeutic modalities provide symptomatic relief and biochemical control, but with no significant survival benefit. The development in the field of molecular pathway responsible for the malignant potential of phaeochromocytoma gives a hope to future therapy. One of the pathways is inhibition of HSP 90 since overexpression of this molecules play an important role in malignant phenotype (30). Therapies targeting angiogenic molecules is the other promising option and sunitinib, a tyrosine kinase inhibitor which acts on these targets shows good results (23,31). Combination of thalidomide (targeting VEGF) and temozolamide seems to have a good response in malignant phaeochromocytoma (32). There are clinical trials assessing the efficacy of other targeted therapies (mTOR inhibitors, HIF inhibitors, prolyl hydroxylase activators and ERBB2 inhibitors) on the horizon (26).

Conclusion

At present, no definite pathological or biochemical markers are available to distinguish malignant phaeochromocytoma from a benign tumour. Currently existing therapeutic modalities mostly offer symptomatic control rather than a cure. The recent development of molecular biology has improved the understanding of
pathways involved in malignant transformation of pheochromocytoma and led to discovery of novel tumour markers. Advances in imaging techniques, nuclear medicine, chemotherapy and radiotherapy provide considerable impact in the management of patients, though limitations still exist. The novel molecular targeted therapies are promising strategies but the development of evidence based approach from large international trials is needed for the successful management.

References

Prevalence of thyroid dysfunction among type 2 diabetic patients attending the Diabetes Clinic, National Hospital of Sri Lanka

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Abstract

Objective: To determine the prevalence of thyroid dysfunction (TD) and to identify risk factors which are associated with TD in Type 2 Diabetic (T2DM) patients attending the Diabetes Clinic, National Hospital of Sri Lanka

Method: A descriptive cross sectional study was carried out at the Diabetes Clinic, National Hospital of Sri Lanka. Study subjects were selected by simple random sampling method and data was collected using an interviewer administered data collection form. TD was assessed by performing 3rd generation TSH and when required FT4/FT3 levels were measured. Statistical analysis was done using Pearson’s Chi-square test, Fisher exact test, Mann Whitney U test and Wilcoxon Rank Sum test and P value < 0.05 was considered as significant.

Results: TD was detected in 83 out of 393 T2DM subjects. The prevalence of TD among study subjects was 21.1% (95% CI: 17.2-25.5%). The most common TD categories were subclinical hypothyroidism (9.4%, 95% CI: 6.7-12.7%) and overt hypothyroidism (6.1%, 95% CI: 3.9-8.9%). Subclinical hyperthyroidism and overt hyperthyroidism were detected only in 5.1% (95% CI: 3.1-7.8%) and 0.5% (95% CI: 0.1-1.8%) of cases respectively. The presence of TD was strongly associated with female sex (p<0.01), advancing age (p<0.01), the presence of goitre (p<0.01) and a positive family history of thyroid disorder among 1st degree relatives (p=0.02). There was no association between the presence of TD and the duration of T2DM, presence of hypertension or chronic complications of DM, type of antidiabetic drugs used, current glycaemic control, body mass index (BMI) and total cholesterol level.

Conclusion: The prevalence of TD was 21.1% and higher prevalence was seen in T2DM patients with female sex, advancing age, presence of goitre and positive family history of thyroid disorder among 1st degree relatives.

Introduction

T2DM has become one of the major non communicable diseases worldwide and the prevalence has risen steadily over the past few decades. In Sri Lanka, the prevalence of T2DM in 2006 was 10.3% and the projected prevalence for the year 2030 is 13.9% (1).

The prevalence of TD among normal subjects varies according to the studied population. In a population-based study done in India on 971 adult subjects, the prevalence of overt and subclinical hypothyroidism was 3.9% and 9.4% respectively. Same study revealed that overt and subclinical hyperthyroidism was present in 1.3% and 1.6% of subjects respectively (2). In the NHANES III study, it was shown that 4.6% of the US population had hypothyroidism (0.3% overt and 4.3% subclinical) and 1.3% had hyperthyroidism (0.5% overt and 0.7% subclinical) (3).

Since both T2DM and TD are common diseases, researches were carried out to explore the possible association between these two endocrinopathies. The first report showing the association between diabetes and TD was published in 1979 (4). Since then several studies from different countries had been done to estimate the prevalence of TD among diabetic patients. The reported prevalence of TD among diabetic patients ranges from 12.3% to 32.4% (5-10). In addition, diabetic women are more frequently affected than men and hypothyroidism (either overt or subclinical) is more common than hyperthyroidism (8). Most of the studies demonstrated

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that the prevalence of TD was higher in diabetic population as compared to general population.

T2DM patients have an increased risk of developing cardiovascular disease which leads to significant mortality and morbidity. Multiple risk factor modification is considered as a key strategy in reducing the cardiovascular risk. TD also (including both subclinical hypothyroidism and hyperthyroidism) has also been linked to increased cardiovascular risk (11). A meta-analysis (including 25,977 participants, 2020 with subclinical hypothyroidism) showed an increased risk of coronary heart disease events and increased risk of cardiovascular and/or all-cause mortality at higher serum TSH concentrations (12). With regard to subclinical hyperthyroidism, a cross-sectional study (in 24,000 older patients) showed that the relative risk of atrial fibrillation in this group was 5.2 compared to euthyroid controls (13). Also a meta-analysis showed a significantly increased risk of all-cause mortality (HR 1.41, 95% CI 1.12-1.79) in patients with subclinical hyperthyroidism (14). Therefore it is reasonable to postulate that the combination of both T2DM and TD may have added cardiovascular risk. So it might be possible to reduce the cardiovascular risk in this subset of T2DM patients by early identification and treatment of TD.

On the other hand, few studies have explored the effects of subclinical thyroid dysfunction on microvascular complications of diabetes. In one cross-sectional study which included 588 subjects, subclinical hypothyroidism was associated with a higher frequency of nephropathy and incident cardiovascular events in patients with T2DM (15). In another study of 1170 subjects, type 2 diabetic patients with subclinical hypothyroidism had a higher prevalence of retinopathy especially the sight-threatening form, when compared with their type 2 diabetic euthyroid counterparts (16).

The relatively high prevalence of both endocrinopathies and adverse impact of TD on diabetes and its complications highlight the importance of screening diabetic patients for TD. However, screening has been currently recommended only in children and adolescents with type 1 diabetes. With regards to adults with T2DM, there is no consensus as to whether screening for thyroid disorders should be mandatory.

To the best of our knowledge no studies have been done to estimate the prevalence of TD in T2DM patients in Sri Lanka. Therefore, the main objective of the study is to determine the prevalence of TD in T2DM patients attending the Diabetes Clinic, National Hospital of Sri Lanka. We also tried to identify any risk factors which are associated with TD in the above population.

Materials and methods

This was a descriptive cross sectional study carried out at the Diabetes Clinic, National Hospital of Sri Lanka. Ethical approval was obtained from the Ethical Review Committee of the Faculty of Medicine, University of Colombo. Patients were recruited to the study after obtaining informed written consent.

T2DM patients who were above the age of 20 years and who had at least 3 previous clinic visits were included in the study. Diagnosis of diabetes was based on American Diabetes Association criteria (17). Simple random sampling method was used to select the subjects for the study. Data was collected through an interviewer administered data collection form. Medical officers at the clinic filled this form by questioning the patient and going through their previous health records. This data included the duration of diabetes, presence of diabetic complications and other comorbid conditions (eg. hypertension, dyslipidaemia), current anti-diabetic medications, past history of thyroid disorder and family history of thyroid disorder among first degree relatives. Patients who reported taking thyroxine, carbimazole, methimazole or propylthiouracil, and those with a history of thyroidectomy or radioactive iodine treatment were identified as having TD.

Anthropometric measurements (height and weight) were measured according to the standard methods using calibrated equipment (18). All these measurements were done by two specially trained diabetes nursing officers at the clinic. Body mass index was calculated by using the following formula – BMI = weight in kgs / (height in meters)².

Blood pressure was recorded as the mean of two consecutive measurements in the sitting position taken 10 minutes apart. Hypertension was defined as BP levels ≥ 140/90 mm Hg or the use of anti-hypertensive drugs. A trained medical officer determined the presence of goitre by palpation method.

Fasting venous blood samples were obtained for glucose, total cholesterol and 3rd generation TSH estimations from all patients who participated in the study. 3rd generation TSH assay was done at the biochemical laboratory of Faculty of Medicine, Colombo using chemilucescence method. Further evaluation with free T4 (free T3 if necessary) was done using the same blood sample in patients who had TSH values which were outside the normal range.

Thyroid dysfunction prevalence for the entire study group and each subcategory were separately identified and 95% confident intervals were determined assuming normal distribution and exact binomial distribution. Group comparisons were performed using Pearson’s Chi-square
test, Fisher exact test, Mann Whitney U test and Wilcoxon rank sum test as appropriate. A P value < 0.05 was considered significant. Analysis was by the SAS System (version 9.0; Cary, USA).

Results

This study included 405 subjects with T2DM who were attending the Diabetic Clinic, National Hospital of Sri Lanka. Data from 393 subjects were used for statistical analysis and 12 patients were excluded because they had not given blood samples for the estimation of TSH. The basic (disease and treatment) characteristics of T2DM study subjects are shown in Table 1. The median age was 57 years and the ages ranged from 25 to 80 years. The median duration of diabetes was 8 years. Among study subjects, 276 (70%) had hypertension and 341 (87%) were on statins. The microvascular and macrovascular complications were present in 123 (31%) and 33 (8%) patients respectively.

Eighty three patients out of 393 diabetic patients had TD. Therefore the prevalence of TD among study subjects was 21.1% (95% CI: 17.2 - 25.5%). The prevalence of TD among females and males were 24.7% and 8.9% respectively. A significantly higher prevalence of TD was observed in females than in males (p<0.01).

The distribution of TD into four main categories was shown in Table 2. The most common TD category was subclinical hypothyroidism (9.4%). This was followed by overt hypothyroidism (6.1%). Subclinical hyperthyroidism and overt hyperthyroidism were detected in 5.1% and 0.5% of cases respectively.

Among 83 patients with TD, only 16 (19.3%) patients were already diagnosed as having some form of TD (clinical hypothyroidism or clinical hyperthyroidism). All the subjects with subclinical TD were newly detected during the study.

In TD group, a goitre was present in 20.5% of subjects. In contrast, patients with normal TSH had goitres only in 5.5%. There was a significant association between TD and the presence of goitre (p=0.01) in T2DM patients. Higher percentage of subjects in TD group (10.8%) gave a positive family history of thyroid disorder among 1st degree relatives when compared with euthyroid group (4.2%) and this association was also statistically significant (p=0.02).

<table>
<thead>
<tr>
<th>Characteristics of T2DM subjects</th>
<th>M(IQR) or N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>57(50-62)</td>
</tr>
<tr>
<td>Gender female</td>
<td>303 (77%)</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>8 (5-13)</td>
</tr>
<tr>
<td>Presence of diabetic complications microvascular</td>
<td>123 (31%)</td>
</tr>
<tr>
<td>Presence of diabetic complications macrovascular</td>
<td>33 (8%)</td>
</tr>
<tr>
<td>Presence of hypertension</td>
<td>276 (70%)</td>
</tr>
<tr>
<td>Statin use</td>
<td>341 (87%)</td>
</tr>
<tr>
<td>Anti-diabetic drug use</td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>366 (93%)</td>
</tr>
<tr>
<td>Sulphonylureas</td>
<td>211 (54%)</td>
</tr>
<tr>
<td>Insulin</td>
<td>133 (34%)</td>
</tr>
<tr>
<td>Acarbose</td>
<td>36 (9%)</td>
</tr>
<tr>
<td>Glitazone</td>
<td>14 (4%)</td>
</tr>
<tr>
<td>Glycaemic control Mean FBS (mmol/L)</td>
<td>6.6 (5.2 - 7.8)</td>
</tr>
<tr>
<td>Glycaemic control Mean PPBS (mmol/L)</td>
<td>7.7 (6.3-10)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>188 (164 - 214)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2 (23.3-28.9)</td>
</tr>
<tr>
<td>Presence of goitre</td>
<td>34 (8.6%)</td>
</tr>
<tr>
<td>Past history of thyroid disorder</td>
<td>35 (9%)</td>
</tr>
<tr>
<td>Family history of thyroid disorder in a 1st degree relative</td>
<td>22 (6%)</td>
</tr>
<tr>
<td>TSH value (mU/L)</td>
<td>1.29 (0.8 - 2.31)</td>
</tr>
</tbody>
</table>

M: Median, IQR: Interquartile range, N: Number, %: Percent
The median age was significantly higher in T2DM patients with TD than those without TD (60 vs 56, p < 0.01). The highest prevalence of TD (38.1%) was observed in patients aged more than 65 years. The prevalence of TD in age groups 50 to 64 years and aged less than 50 years were 20.6% and 12.3% respectively.

Detection of TD was not associated with duration of diabetes (p=0.42), presence of hypertension (p=0.20), or recent glycaemic control (p=0.17 and 0.12) based on FBS and PPBS respectively. Also the presence of microvascular (p=0.31 for retinopathy, p=0.80 for nephropathy and p=0.28 for neuropathy) and macrovascular complications (p=0.76 for IHD) of diabetes wasn’t significantly associated with TD among our T2DM patients. There were no patients with TD who had either CVA or PVD. Also this study demonstrated that there was no association between the presence of TD and being on different types of anti-diabetic medications (p=0.26 for metformin, p=0.17 for sulphonylureas and p=0.81 for insulin, p=0.49

### Table 2. Prevalence of thyroid dysfunction according to main categories

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of patients</th>
<th>Prevalence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overt hypothyroidism</td>
<td>24</td>
<td>6.1% (3.9-8.9)</td>
</tr>
<tr>
<td>Subclinical hypothyroidism</td>
<td>37</td>
<td>9.4% (6.7-12.7)</td>
</tr>
<tr>
<td>Overt hyperthyroidism</td>
<td>2</td>
<td>0.5% (0.1-1.8)</td>
</tr>
<tr>
<td>Subclinical hyperthyroidism</td>
<td>20</td>
<td>5.1% (3.1-7.8)</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>21.1% (17.2-25.5)</td>
</tr>
</tbody>
</table>

### Table 3. Clinical and laboratory characteristics of T2DM patients with thyroid dysfunction 83 (21.1%) vs without thyroid dysfunction 310 (78.9%)

<table>
<thead>
<tr>
<th>Characteristics of T2DM subjects</th>
<th>Diabetic subjects without thyroid dysfunction</th>
<th>Diabetic subjects with thyroid dysfunction</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M (IQR) or N(%)</td>
<td>M (IQR) or N(%)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>60 (55-65)</td>
<td>56 (49-61)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>75 (90.4%)</td>
<td>228 (73.5%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Male</td>
<td>8 (9.6%)</td>
<td>82 (26.5%)</td>
<td></td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>8 (6-14)</td>
<td>8 (5-13)</td>
<td>0.42</td>
</tr>
<tr>
<td>Presence of goitre</td>
<td>17 (20.5%)</td>
<td>17 (5.5%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Presence of hypertension</td>
<td>63 (75.9%)</td>
<td>213 (68.7%)</td>
<td>0.20</td>
</tr>
<tr>
<td>Anti-diabetic drug use:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>75 (90.4%)</td>
<td>291 (93.9%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Sulphonylureas</td>
<td>39 (47.0%)</td>
<td>172 (55.5%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Insulin</td>
<td>29 (34.9%)</td>
<td>104 (33.5%)</td>
<td>0.81</td>
</tr>
<tr>
<td>Acarbose</td>
<td>7 (8.4%)</td>
<td>29 (9.3%)</td>
<td>0.80</td>
</tr>
<tr>
<td>Glitazone</td>
<td>4 (4.8%)</td>
<td>10 (3.2%)</td>
<td>0.49</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0 (23.2-28.7)</td>
<td>26.8 (23.9-29.2)</td>
<td>0.31</td>
</tr>
<tr>
<td>Glycaemic control:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBS (mmol/L)</td>
<td>6.2 (5-7.8)</td>
<td>6.5 (5.65-7.55)</td>
<td>0.17</td>
</tr>
<tr>
<td>PPBS (mmol/L)</td>
<td>7.7 (6.2 - 9.8)</td>
<td>8.2 (6.8 - 10.5)</td>
<td>0.12</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>184 (164-212)</td>
<td>196 (160-224)</td>
<td>0.35</td>
</tr>
<tr>
<td>TSH value (mU/L)</td>
<td>1.2 (0.85-1.86)</td>
<td>4.4 (0.26-6.56)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Discussion

Our study showed that the prevalence of TD among Sri Lankan T2DM patients attending outpatient clinic was 21.1%. Previous studies from various regions of the world, assessing the prevalence of TD among diabetic patients have shown a wide range of values. However, one consistent observation across all these studies was that there was a higher prevalence of TD in diabetic subjects when compared with normal population. A study from Spain which included 318 subjects reported 32.4% prevalence of TD among type 2 diabetic patients (5). Another Italian study (6) also showed a higher prevalence (31.4%) of TD in diabetic population. In contrast, a study by Perros et al. (7) from Scotland found that the prevalence of TD among type 2 diabetic patients was 13.4%. A Greek study (8) demonstrated a TD prevalence of 12.3% among their diabetic patients. In another study by Akbar et al (9) in Saudi Arabia the association between thyroid dysfunction and type 2 DM was investigated and TD was found in 16% of diabetics. When the data from South Asian countries were considered, an Indian study showed a prevalence of 31.2% (10). However, the major drawback of this study was the lack of randomization which can lead to the overestimation of prevalence. However, we also found a relatively higher prevalence of TD among our T2DM patients after proper randomization.

Although the exact cause for the higher prevalence of TD among our T2DM population was not known, one of the possible major contributory factors might be the higher degree of insulin resistance. Higher levels of circulating insulin associated with insulin resistance have shown a proliferative effect on thyroid tissue resulting in larger thyroid size with increased formation of nodules (19,20) which can result in TD.

In our T2DM patients, the most common TD categories were subclinical hypothyroidism (9.4%) and overt hypothyroidism (6.1%). Both overt and subclinical hyperthyroidism were seen less frequently. Most of the other studies (6,10) also have shown similar pattern of TD among diabetic patients. Community based studies among normal population in India (21) and USA (3) also demonstrated a similar trend of TD. In general, the data indicate that the presence of T2DM only increase the prevalence without changing the pattern of TD.

It is a well-known fact that both hypothyroidism and hyperthyroidism are seen more commonly in females than in males. In this study, the prevalence of TD among females was significantly higher (24.7% vs 8.9%) and female sex is a risk factor for having TD among T2DM patients. In our study, the prevalence of goitre among study subjects was 8.6%. A community based Sri Lankan study showed that the prevalence of goitre was 6.5% (22). Increased thyroid volume and nodularity due to high insulin levels secondary to insulin resistance and higher median age (57 vs 38 years) may be the most probable explanations for the slightly increased prevalence of goitre observed in our study. As with normal population, the presence of a goitre or family history of thyroid disorder in a 1st degree relative is a risk factor for having TD among T2DM patients.

Majority of patients with overt hypothyroidism and significant number of patients with subclinical hypothyroidism, have high serum total and LDL cholesterol concentrations (23). In contrast, patients with hyperthyroidism have changes in lipid metabolism generally opposite to those described above for hypothyroidism. Serum total and LDL cholesterol concentrations tend to be low (24). In keeping with this observation, hypothyroid subjects (overt/subclinical) in our study had a statistically significant higher median total cholesterol concentration than their hyperthyroid counterparts (200 vs 164 mg/dL, p=0.02).

Several studies have shown that TD was associated with increased cardiovascular risk and mortality (11). However the data from our study indicated that the presence of ischaemic heart disease was not considerably higher in TD group when compared with euthyroid diabetic patients (8.4% vs7.4%, p=0.76).

Conclusion

This study highlights the high prevalence (21.1%) of TD among T2DM patients followed up at the Diabetic Clinic, National Hospital of Sri Lanka and the strong association of TD with female sex, advancing age, presence of goitre and a positive family history of thyroid disorder among 1st degree relatives. Based on this data, we emphasize the importance of the screening for TD in selected patients with T2DM. Also this study would provide baseline data to plan management strategies for the above group of patients and to conduct future research on TD among Sri Lankan T2DM patients in a national level.
Acknowledgments

The authors would like to acknowledge the staff of the Diabetic Clinic, National Hospital of Sri Lanka, the staff of Biochemical Laboratory of Faculty of Medicine, University of Colombo for their support and all the subjects who participated in the study. Funding for this study was provided by the Abbot Laboratories.

References


Abstract

**Introduction:** Use of growth hormone therapy (GHT) in adolescents is not common in Sri Lanka. In this study we aimed to assess the response to GHT in adolescents in our setting presenting with short stature.

**Materials and methods:** This was an observational study carried out at the University Medical Clinic, National Hospital of Sri Lanka. GHT was used for those with growth hormone deficiency (n=15), Turner syndrome (n=5) and Prader Willi syndrome (n=1). All were monitored with anthropometric measurements, IGF-1 and observed for side effects.

**Results:** Among the 21 adolescents, 15 were males (71.4%). Mean age, height, weight at presentation were 15.0 (10.4-19.1) years, 138.6 (+7.6) cm and 38.4 (+13.0) kg respectively. Low IGF-1 was found in 16 (76.1%). IGF1 was normal in Turner patients. Impaired response for ITT was seen in 13 (81.3%; n=16). In one patient it was discontinued due to lack of patient cooperation.

Follow up period and mean growth velocity were 2.7 – 35.6 months and 7.1 (+3.7) cm/year respectively. In the group with isolated GHD (n=13) significant negative correlation between initial bone age and growth velocity was found but that of initial chronological age and height was not significant.

Girls with Turner syndrome showed a mean height velocity of 5.9cm/year with therapy.

Side effects were detected in 3 (14.9%) patients which were diabetes mellitus (n=2) and carpal tunnel syndrome (n=1).

**Conclusion:** Growth hormone replacement therapy is useful in achieving satisfactory height gain in adolescents with short stature due to GHD, Turner syndrome and Prader Willi syndrome and is usually a safe treatment.

**Key words:** growth hormone, short stature, adolescents

**Introduction**

Short stature is a distressing problem for the affected children or adolescents or their families. It is defined as height more than 2 standard deviations below the mean or below the 3rd percentile for age, sex and ethnicity (1). Genetic and familial factors, intrauterine factors affecting the intrauterine growth, environmental factors such as malnutrition, chronic illnesses and endocrine disorders have an important place among the many determinants of growth. However only 20% will have an identifiable pathological cause while almost 80% accounts for constitutional delay (1).

Prevalence of growth hormone deficiency (GHD) is estimated to be ranging from 1:4000 to 1:10,000 worldwide (2), but data on the prevalence of short stature or growth hormone deficiency in Sri Lanka is limited. The first report on short stature in Sri Lanka was by de Mel et al in 1987 which reported a prevalence of 5.3% among Sri Lankan children (3). A study carried out in 1991 in Sri Lanka showed that out of 16,001 children screened for short stature 172 were identified as short out of which 12 children were diagnosed to have growth hormone deficiency by provocative testing (4).

Growth hormone deficiency commonly occurs due
to idiopathic isolated GHD and less frequently due to other pathological causes (5). Anthropometric measurements, provocative testing, measurement of IGF1 and IGF binding protein-3, radiographic assessment of bone age and cranial MRI (6) are helpful in the diagnosis of growth hormone deficiency in a child with persistently subnormal growth with no other identifiable cause (6,7).

The recommendations for recombinant growth hormone therapy are growth hormone deficiency, Turner syndrome, Prader-Willi syndrome, chronic renal insufficiency, children with intrauterine growth restriction who have not reached a normal height range by the age of 2 years, children with severe idiopathic short stature (height >2.25 SD below the mean height) who are unlikely to catch up in height and short stature due to homeobox-containing gene (SHOX) deficiency (5, 7). In addition to these, growth hormone therapy is indicated in adults with growth hormone deficiency and adults with wasting. Treatment should be monitored by a specialist endocrinologist for both children and adults (7).

In this report we present data on 21 adolescents attending our endocrine clinic who were treated with growth hormone therapy for short stature.

Materials and methods

Study population and study setting

21 adolescents who were treated with recombinant growth hormone therapy for short stature attending the Endocrine Clinic of the University Medical Unit of National Hospital of Sri Lanka were studied.

Study period

Study was carried out between February 2010 and June 2013.

Procedure

Informed written consent was provided by the patients or guardians.

Patients were selected for growth hormone treatment by a consultant endocrinologist. Anthropometric measurements (height and weight) were recorded and clinical and biochemical investigations (renal function tests, liver function tests, fasting blood glucose, haemoglobin levels) were carried out to exclude chronic illnesses. All patients underwent serum cortisol, serum TSH, free T4 and when appropriate serum FSH, LH and testosterone measurements to assess co-existing endocrinopathies.

All patients were investigated with IGF-1 levels and those with low IGF underwent ITT. Priming with sex steroids were carried out prior to provocative testing in adolescents with pubertal delay (girls aged 11.5-12 years and boys aged 13-13.5 years with no evidence of puberty or only initial signs). ITT was carried out in patients with Turner syndrome only if there was evidence of pituitary insufficiency.

Bone age was assessed using skeletal x-rays of left wrist and hand. MRI was carried out in all patients with growth hormone deficiency or suspected intracranial abnormality.

Patients with growth hormone deficiency were treated with human recombinant growth hormone 0.3 mg/kg/week subcutaneously and dose adjustments were made according to the response. Dose for Turner syndrome was 0.375 mg/kg/week (8) and for children with Prader Willi syndrome initial dose of 0.5mg/m² was given and dose gradually increased to 1mg/m² with time (9).

Serial anthropometric measurements were monitored during each clinic visit. IGF-1 levels, bone age and other relevant investigations were periodically performed. Serum TSH was measured intermittently as growth hormone therapy may unmask hypothyroidism (7, 10). Adverse effects of growth hormone therapy were noted. Further treatment with growth hormone was discontinued if there was no increase in growth rate or static serum IGF concentration was detected within the first 6 to 12 months in a compliant patient receiving an appropriate dose of GH (7).

Results

Sociodemographic variables

Among the 21 adolescents who were observed, 15 were males (71.4%). Mean age at presentation of all patients was 15.1 (±2.0) years and that of bone age was 11.1 (±2.2) years. There was no significant difference in the age of presentation between males and females (p=0.08). Mean height and weight at presentation were 138.6 (±7.6) cm and 38.4 (±12.9) kg. Follow up period was 2.7-35.6 months (mean=17.6 months). Characteristics are summarized in Table 1.

Aetiology

Aetiology of short stature in the patients was identified to be as mentioned in Table 2.

Biochemical findings

IGF1 levels were low when compared with age appropriate ranges in 16 (76.1%) patients. All Turner patients had normal IGF-1 levels. In the 16 patients with low IGF-1, ITT was carried out and a failed response was found in 13 (81.3%) and 2 (12.5%) showed normal growth hormone response to stimulation. ITT was discontinued in one patient due to lack of patient cooperation.
### Table 1. Sociodemographic variables of different groups of adolescents with short stature in the cohort

<table>
<thead>
<tr>
<th></th>
<th>All - Male (n=15)</th>
<th>Isolated growth hormone deficiency - Male (n=10)</th>
<th>Turner syndrome - Male (n=5)</th>
<th>Prader Willi syndrome - Female (n=1)</th>
<th>Panhypopituitarism - Male (n=3)</th>
<th>Growth hormone deficiency and hypothyroidism - Male (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>139.6</td>
<td>137.8</td>
<td>134.3</td>
<td>146</td>
<td>143.3</td>
<td>140</td>
</tr>
<tr>
<td>SD*</td>
<td>8.2</td>
<td>7.7</td>
<td>4.3</td>
<td>0</td>
<td>12.1</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>38.8</td>
<td>32.8</td>
<td>35.6</td>
<td>80</td>
<td>40.7</td>
<td>52</td>
</tr>
<tr>
<td>SD*</td>
<td>15.0</td>
<td>9.0</td>
<td>5.9</td>
<td>9.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kgm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>19.6</td>
<td>17.1</td>
<td>19.8</td>
<td>37.5</td>
<td>19.8</td>
<td>26.5</td>
</tr>
<tr>
<td>SD*</td>
<td>6.5</td>
<td>3.8</td>
<td>3.7</td>
<td>3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of presentation (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>14.6</td>
<td>14.5</td>
<td>16.4</td>
<td>10.4</td>
<td>17.0</td>
<td>12.8</td>
</tr>
<tr>
<td>SD*</td>
<td>2.1</td>
<td>1.5</td>
<td>0.9</td>
<td>1.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Sample standard deviation

### Table 2. Aetiology of short stature of adolescents who were initiated on growth hormone therapy

<table>
<thead>
<tr>
<th>Cause</th>
<th>Number of patients diagnosed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic</td>
<td></td>
</tr>
<tr>
<td>Turner syndrome without other endocrinopathy</td>
<td>3 (14.3%)</td>
</tr>
<tr>
<td>Turner syndrome and hypothyroidism</td>
<td>2 (9.5%)</td>
</tr>
<tr>
<td>Prader Willi syndrome</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td>Endocrine</td>
<td></td>
</tr>
<tr>
<td>Isolated growth hormone deficiency</td>
<td>11 (52.4%)</td>
</tr>
<tr>
<td>Hypothyroidism and growth hormone deficiency</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td>Panhypopituitarism</td>
<td>3 (14.3%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>21 (100%)</strong></td>
</tr>
</tbody>
</table>
**Imaging**

All patients confirmed with GHD were investigated with MRI scan of the pituitary. MRI was abnormal in 3 patients. One patient was diagnosed as pituitary hypoplasia with probable empty sella syndrome while the others were diagnosed with pituitary microadenoma. In those with abnormal MRI there was no significant difference between the initial height (143.3 (±12.1) cm vs. 137.3 (±7.2) cm; p=0.26), age at presentation (17.0 (±1.8) years vs. 14.7 (±2.0) years; p=0.10) and growth velocity achieved (5.9 (±3.7) cm/year vs. 8.03 (±4.1) cm/year; and p=0.43) when compared with those with normal MRI.

**Growth velocities**

In the entire sample, the mean growth velocity among males (n=15) was 8.3 (±4.0) cm and among females (n=6) was 5.5 (±1.9) cm.

In Turner patients (n=5) mean growth velocity achieved was 5.8 (±1.9) cm/year. In those with isolated growth hormone deficiency there was no significant correlation between growth velocity and age at initiation of therapy (r = -0.28, p=0.392), height at initiation of therapy (r=-0.45, p=0.162) and mid parental height, which indicates the genetic growth potential of the patient (r = 0.171; p=0.614). But bone age at presentation had a significant negative correlation with growth velocity (r = -0.641; p = 0.046) in the same group.

Along with growth hormone, testosterone therapy was initiated in 2 male patients out of the 15 males. They were able to achieve a mean growth velocity of 2.3 cm/year which was significantly low compared to the rest without testosterone therapy (p=0.006).

**Prader Willi syndrome**

The patient with Prader Willi syndrome showed impaired ITT and low IGF-1 levels at the initial assessment.

The child with Prader Willi syndrome lost 11 kg during initial 3 months of growth hormone therapy, following which he was diagnosed to have diabetes mellitus. He was initiated on insulin therapy and optimal glycaemic control was achieved. His weight was stable there onwards. His height increased 4 cm over the 9 months of observation.

**Panhypopituitarism**

The adolescents with panhypopituitarism showed a mean height velocity of 5.9 cm/year (±3.7)

**Final height**

The table below depicts the percentiles and standard deviation of the patients’ initial and final height.

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Age (years)</th>
<th>Initial height (cm)</th>
<th>Centile</th>
<th>SD</th>
<th>Duration of therapy (months)</th>
<th>Final height (cm)</th>
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Table 3. Percentile and standard deviation of patients’ initial and final height
Side effects

During growth hormone therapy 3 (14.9%) patients developed side effects. One patient was diagnosed with carpal tunnel syndrome 23 months after commencement of therapy. Two patients were diagnosed with diabetes mellitus after 5 months and 3 months of commencement of therapy and one of them was the patient with Prader Willi syndrome.

Discussion

Growth hormone was initially extracted from pituitary of cadavers in the 1950’s but the safety was an issue due to diagnosis of Creutzfeldt-Jakob disease (11). Recombinant growth hormone came in to use in early 1980’s which was safer, and provided large amounts of growth hormone according to the demand (11).

In our patients mean age of presentation of growth hormone deficiency was 14.9 years. Severe growth hormone deficiency due to complete absence of growth hormone presents at a younger age, usually before 3 years (13). Those with late onset growth hormone deficiency or those with milder forms present at an older age (13). But delay in presentation in our context could also be attributed to lack of parents’ knowledge in reaching medical advice, attributing short stature in their children as a normal phenomenon and poor growth monitoring at child welfare clinics and school health programmes.

Same dose of growth hormone is found to be less effective in accelerating the growth velocity after 6-12 months of therapy. Catch up growth can be obtained with increasing the replacing doses of growth hormone (12). Therefore we altered the dose, titrating it with the IGF values in the upper normal range and our patients were able to achieve a satisfactory increase in height.

There were 2 children who had low IGF-1 levels but normal ITT response. This kind of a picture is seen in growth hormone resistance but these adolescents did not show severe growth retardation which is seen in some forms of growth hormone resistance. These two adolescents were given a trial of growth hormone therapy to which they showed a good response with a growth velocity of 9.12cm/year. Moreover there is growing debate on the need of ITT in diagnosing growth hormone deficiency as stimulation tests are found to be highly variable, inaccurate and non-physiological (14-16). Therefore, serum IGF-1 levels along with the probable clinical features are considered to be a better marker (14-16).

At the end of mean follow up period of 1.3 (±0.8) years our patients with isolated growth hormone deficiency were able to achieve a growth velocity of 8.6 cm/year (±3.0 cm/year). Studies carried out in other countries have shown mean growth rates of 8.4-9.5 cm/year in the first year of treatment [17]. Genetic potential of the Caucasians would have contributed to the differences.

Several studies have reported a significant negative relationship between the linear growth response following GH therapy and chronological age, height, bone age. But in our adolescents we were able to establish a significant negative correlation between growth velocity and the bone age only.

Our patients did not show significant difference in age and height at presentation and growth velocity between those with normal and abnormal MRI scans. But according to Coutant et al (18) patients with growth hormone deficiency and MRI abnormalities are found to have severe short stature at diagnosis, younger age at diagnosis and significantly high catch up growth in response to treatment when compared with those with normal MRI scans. Despite this, when growing in to adulthood, studies show that > 63% of those with normal MRI scans have normalized their growth hormone secretary status while those with abnormal MRIs tend to have persistent growth hormone deficiency.

Turner syndrome is found to be a common cause for short stature among girls. Normal IGF1 levels were found in 4 of the 5 girls with Turner syndrome in our adolescents. Lebl J et al (19) states that the pathogenesis of growth failure in Turner syndrome could be due to reduced sensitivity to IGF1 or its reduced activity. In that study, IGF-1 levels done in 78 untreated Turner patients revealed low normal IGF-1 levels and treatment with growth hormone had lead to supraphysiological levels of IGF-1. In the same study, there had not been additional increase in IGF1 with addition of oestradiol to the growth hormone regimen. In our patients the IGF1 levels increased and maintained at an upper normal range in response to growth hormone therapy and were able to achieve a mean height velocity of 5.8cm/ year. Several studies have shown that at the end of treatment patients are able to achieve an increase in height of 5-10 cm but the growth improvement tends to decrease with time (20). Duration of treatment in Turner, though those who are treated are taller than untreated females, is still controversial because of the high cost and the final height is found to be below normal range (20).

We had one patient with Prader Willi syndrome who had biochemical evidence of growth hormone deficiency. It was found that more than half of Prader Willi children will have biochemical evidence of GHD (21,22). Obesity, poor growth, and hypotonia in these children are associated with abnormal body composition resembling a GH-deficient state (23). Recommended dosage for these children differs from the others. Continuation of growth hormone therapy is necessary until risks outweigh the benefits. Severe obesity was considered as exclusion criteria in children with Prader Willi syndrome for growth
hormone therapy. Though our child was obese (BMI = 37.53kg/m²) he did not fit into ‘severely’ obese criteria. According to the consensus guidelines severe obesity is defined if a child is with a BMI of >95th centile and manifests complications of obesity such as sleep apnoea, non-alcoholic fatty liver disease or abnormal carbohydrate metabolism (9). Evaluation for diabetes risk should be carried out in children more than 12 years of age or in those with a family history of diabetes (9). Since our child did not fit into the above criteria prior screening was not done. But within 3 months of therapy he developed hyperglycaemic symptoms and we started insulin regimen and continued with growth hormone therapy.

Continuation of growth hormone into adulthood is still controversial. Most children outgrow from growth hormone deficiency when they reach adulthood. Transition period from adolescence to adulthood involves achieving the adult height, peak bone mass, adult body composition and reproductive maturity so continuation therapy in deficient individuals is important (23). Thus discontinuation of treatment in deficient individuals may lead to deleterious effects. Persistent growth hormone deficiency can be best predicted (PPV 100%) in those with organic hypothalamic pituitary disorder or ≥2 additional pituitary hormone deficiencies. In those with idiopathic growth hormone deficiency IGF-1 levels below -5.3 SD measured ≥6 weeks after completion of growth hormone treatment (PPV 100%) is also a good predictor (24).

Therefore, in patients with pathologic disease a stimulation test to confirm growth hormone deficiency is required in the transition period. The appropriate dose of growth hormone during this period is controversial as secretion of the hormone is lower than during puberty but higher than during adulthood (23). Still our patients were not assessed for changes occurring during the transition period.

Since growth hormone is given as once a day subcutaneous injection, it is difficult to produce the normal pulsatile pattern of secretion. Despite this, the growth hormone therapy in deficient individuals leads to improved generalized wellbeing, improved body composition with increased lean body mass and reduced fat deposition. It improves bone mineral density, and lipid profile. In order to assess effects of therapy on the cardiovascular disease, bone fracture and the quality of life, it will require many years of growth hormone replacement (23).

Conclusions

In this study we were able to demonstrate an increase in growth velocity and IGF-1 levels in patients with growth hormone deficiency by growth hormone replacement. However lack of growth charts appropriate for Sri Lankan population is a main disadvantage as growth monitoring is difficult in growth charts for Caucasians. Also focusing the attention on early initiation of growth hormone therapy in Turner syndrome is important for clinicians. As a whole, growth hormone therapy is usually safe and is useful in gaining satisfactory height gain in adolescents.

References


Type 2 diabetes (T2DM) is a heterogeneous disorder. It affects the young, middle aged and elderly. Some patients with T2DM are obese and some are lean. A category of individuals in these groups posses a cluster of cardiovascular risk factors named as metabolic syndrome comprising dyslipidemia, high blood pressure and central obesity. All these features highlight the heterogeneity and different phenotypes of T2DM.

Hyperglycemia is the main therapeutic target in the management of T2DM. Over many decades, a number of oral hypoglycemic agents (OHA) have been introduced to control hyperglycemia in this disorder. Professional organizations formulated guidelines to use OHAs in T2DM. Most of these guidelines stipulate a vertical approach in selecting and adding OHAs. With the availability of more OHAs and better understanding of the heterogeneity of diabetes, there is a paradigm shift in the guidelines in the management of hyperglycemia in diabetes. The most recently advocated approach endorses an individualized treatment based on the age, impact of weight gain or necessity of weight loss, target glycemic control, risk of hypoglycemia, cost and patient safety in selecting the most appropriate OHA for a given patient with T2DM. This article aims to address some clinically relevant issues related to the individualized therapy in T2DM.

Metformin as the first line OHA

Metformin is one of the oldest of the OHAs to be used to control hyperglycaemia in T2DM. The findings of the United Kingdom Prospective Diabetes Study (UKPDS) revealed that it is effective in reducing mortality when used in obese patients with T2DM over sulphonylureas (1). This observation lead to recommendation of metformin as the first choice of OHA for the newly diagnosed, obese subjects with T2DM. Most recently advocated approach endorses an individualized treatment based on the age, impact of weight gain or necessity of weight loss, target glycemic control, risk of hypoglycemia, cost and patient safety in selecting the most appropriate OHA for a given patient with T2DM. This article aims to address some clinically relevant issues related to the individualized therapy in T2DM.

The most appropriate agent after failure of metformin monotherapy

Type 2 diabetes is a progressive disease. Although metformin is used as the first-line therapy, most, if not all patients require additional agents to achieve the recommended glycemic control with increasing duration of the disease. According to data from the National Health and Nutrition Survey in the United States, the most common oral medications used to treat diabetes after metformin monotherapy changed from sulfonylureas in 1999 to 2000 to glitazones in 2003 to 2004 (3). Introduction of incretin based therapies namely glucagon like peptide (GLP) analogues and dipeptidyl peptidase-4 (DPP-4) inhibitors during the past few years to treat hyperglycemia in T2DM has posed many challenges to this practice.

A recent meta-analysis using PubMed and the Cochrane central register written in English through December 2011 including 39 randomized controlled trials involving 17,860 patients with T2DM on different regimens of hypoglycemic therapy has posed new challenges to the current practice (4). According to their findings, GLP analogues resulted in greater decrease in A1c levels compared with sulfonylureas (-0.20%; 95% confidence interval [CI], -0.34% to -0.04%), glinides (-0.31%; 95% CI, -0.61% to -0.02%), glitazones (-0.20%; 95% CI, -0.38% to -0.00%), α-glucosidase inhibitors (-0.36%; 95% CI, -0.64% to -0.07%), and dipeptidyl peptidase 4 inhibitors (DPP-4 inhibitors; -0.32%; 95% CI, -0.47 to -0.17%), and resulted in A1c levels comparable to basal insulin and biphasic insulin.

The authors found that sulfonylureas, glinides, basal insulin, and biphasic insulin treatments were associated with an increased risk for hypoglycemia compared with placebo. Patients receiving sulfonylureas, glinides,
glitazones, basal insulin, and biphasic insulin gained weight, and patients receiving α-glucosidase inhibitors and GLP analogues lost weight.

These findings and their apparent therapeutic efficacy and safety have promoted incretins as the potential agents after metformin mono therapy and some professional bodies have included them as the second line therapy in their therapeutic algorithms to control hyperglycemia in T2DM. The main drawback of GLP analogues however is that, like insulin it needs to be given as subcutaneous injections (either daily or extended release preparations once weekly). Not many patients with T2DM would willingly switch over to an injectable preparation when other oral therapies are available to control hyperglycemia. As a substantial proportion of patients with T2DM in the South East Asian region are lean, they would not benefit from weight reducing effects of GLP analogues.

The oral form of incretin, DPP-4 inhibitors or gliptins does not have the same potential to reduce HbA1C as GLP analogues. A recent study revealed an increased incidence of heart failure related hospital admissions among patients treated with saxagliptin therapy and as expected saxagliptin had no effect on the cardiovascular mortality (5). However findings of this single trial alone is not adequate to disregard the value of DPP-4 inhibitors. Therefore, recommendation of either form of incretins, injectable or oral, as the most appropriate agent to treat T2DM after metformin mono therapy should await results of the ongoing long term studies. With available evidence, the preferential use of GLP -1 analogue should be limited to selected patients with T2DM in whom weight loss and risk of hypoglycemia are major concerns. Similarly, until evidence on favorable long cardiovascular outcomes is available, gliptins can only be used as alternatives, not as preferred therapy to currently widely used OHAs such as sulphonylureas and glitazones. The 2012 position statement by the American College of Clinical Endocrinologists (ACCE) on the choice of hypoglycemic therapy has endorsed gliptins only as an alternative agent after metformin therapy in their therapeutic algorithm (6).

Place of glitazones amidst safety concerns

Glitazones (thiazolidinediones) have been in use as an OHA for more than a decade. But they have caused considerable controversy since they were introduced into the management of patients with T2DM. Until recently, there were two glitazones licensed for use in the treatment of type 2 diabetes: rosiglitazone and pioglitazone. But rosiglitazone has been withdrawn from the market in 2011 because of concerns about cardiovascular safety, mainly heart failure. But recent reports indicate that rosiglitazone may be reintroduced with safety precautions in those with incipient heart failure. Although there is no evidence to show similar cardiovascular adverse effects of pioglitazone, several reports on its association with fluid retention, fractures and bladder cancer have initiated a debate on its risks vs benefits as a therapeutic agent in T2DM.

Recently, British Medical Journal published a retrospective cohort study including 600 general practices in the United Kingdom among 115, 727 individuals with type 2 diabetes who were newly treated with oral hypoglycaemic agents between January 1988 and December 2009 (7). It was revealed that use of pioglitazone was associated with an increased rate of bladder cancer (rate ratio 1.83, 95% confidence interval 1.10 to 3.05). The rate increased as a function of duration of use, with the highest rate observed in patients exposed for more than 24 months (hazard ratio (HR) -1.99, CI -1.14 to 3.45) and in those with a cumulative dosage greater than 28,000 mg (HR -2.54, CI -1.05 to 6.14).

The US Food and Drug Administration issued a warning on use of the drug. It is contraindicated in patients with symptomatic heart failure and advised to be used with caution in women with high risk for fractures. France and Germany had already suspended its use.

Although there are few concerns with safety, as an OHA, pioglitazone has a special place in management of patients with T2DM in the local setting. Its insulin sensitising effect is useful in treating patients with severe insulin resistance (IR) especially the category of patients with non-alcoholic fatty liver (NAFLD) and polycystic ovary syndrome (PCOS), both common accompaniments of T2DM. As the third or fourth OHA for those taking maximal doses of metformin and sulphonyluria with or without alpha glucosidae inhibitors, it delays the initiation of insulin for a considerable period. This property of pioglitazone avoids the need for insulin injections which majority of patients fail to adhere to long term. ADOPT (A Diabetes Outcome Progression Trial) demonstrated that initial monotherapy with glitazone provided superior durability of glycemic control compared with metformin and glibenclamide in patients with recently diagnosed type 2 diabetes (8).

These positive features of pioglitazone useful in managing patients in the local setting are too good to be overlooked in taking the decision to stop glitazones in patients who maintain satisfactory glycemic control. Therefore, in the absence of risk factors for bladder cancer, pioglitazone can be continued with caution in a selected category of patients with T2DM without heart failure or high risk for bone fractures.

Therapeutic options for OHA failure

The progressive nature of T2DM necessitates regular dose escalation and addition of newer OHAs to maintain optimal glycemic control with increasing duration of the
disease. But in most patients, optimal glycemic control cannot be maintained even with maximal doses of all the available and tolerable oral agents 5-10 years after the diagnosis of T2DM. This stage is called OHA failure in T2DM. Until recently initiation of insulin was the only option available to control hyperglycemia in these patients. Availability of GLP analogues has provided another therapeutic option to manage OHA failure. However presence of some residual beta cells is necessary for the function of GLP analogues. Both insulin and GLP analogues need to be administered as subcutaneous injections, the notable advantage of GLP analogues over insulin includes the lack of hypoglycemic risk and associated weight loss. Recently introduced basal insulin has a comparatively lesser risk of hypoglycemia than previous insulin regimens, but weight gain is a considerable concern when insulin of any type is commenced in already overweight or obese patients with OHA failure.

Clinicians should take many factors into consideration before initiating either insulin or GLP analogues especially for the elderly patients with OHA failure. Evidence from clinical trials (ACCORD, VADIT) reveals that attempts to intensify glycemic control in elderly and those with pre-existing cardiac disease are associated with an increased risk of death (9,10). Basal insulin and GLP analogues are costly and their cost effectiveness should be given a thought when commencing in the poor resource setting. Most elderly patients find it difficult to use daily, self-injectable preparations and the long-term adherence of such therapy is doubtful. Because of all these concerns, professional associations have recently laid down less stringent glycemic targets for elderly patients with diabetes.

For younger patients with OHA failure, choice of either type of injection (insulin or GLP analogues) should be decided based on individual needs. Beta cell preserving effects of GLP analogues may be useful for younger patients if administered early in the disease. The obese would also benefit from weight losing property of GLP analogues. Insulin would be less costly and more appropriate for lean patients with OHA failure as it would cause some gain in weight and self-satisfaction for those with severe emaciation. When once daily injection and appropriate combinations of OHAs fail to achieve the recommended glycemic target, switching over to a more frequent insulin regimen should be considered.

In conclusion, clinicians should give due consideration to individual factors in different patients with T2DM when choosing the most appropriate agent to manage hyperglycemia while adhering as much as possible to the evidence based guidelines. Strict adherence to guidelines alone may sometimes cause more harm to an individual patient.

References

Hypothyroid pseudoacromegaly

S D L P Subasinghe¹, W S T Swarnasri², G A Ratnatilake³, N P Somasundaram⁴


A 70 year old male presented with 2 months history of headache with impaired peripheral vision and 6 months history of progressive enlargement of face, hands and feet together with weight gain, constipation and lethargy. He had acromegalic features (Figure 1) and constricted temporal visual fields. Increased heel pad thickness compatible with acromegaly was noted on X-ray. He was found to have homogenous enlargement of the pituitary gland suggestive of a macroadenoma on Magnetic Resonance Imaging (MRI) of brain. Endocrine studies showed TSH >75 μIU/ml (ref. 0.4-4); Free T₄ 0.36 ng/dL (ref. 0.89-1.76). A 75g oral glucose tolerance test showed normal suppression of GH level. The constricted temporal visual fields were explained by glaucoma. The patient was started on levothyroxine 100μg daily and his clinical condition, biochemical parameters as well as the secondary pituitary hyperplasia is currently responding well to treatment (Figure 2A and 2B).

The elevated thyrotropin-releasing hormone (TRH) level due to the absence of negative feedback seen in primary hypothyroidism, leads to thyrotrh hyperplasia causing pituitary enlargement, which is a known but rare occurrence (1). Since MRI alone is unable to reliably differentiate between a thyroid stimulating hormone (TSH)-secreting pituitary adenoma and hypothyroidism-induced pituitary hyperplasia dynamic endocrine tests such as blunted TRH stimulation test, elevated alpha subunit concentration and elevated alpha subunit concentration/TSH ratios (2) as well as repeat pituitary MRI after a brief thyroxine trial are used to provide confirmation in similar cases (3). Pituitary hyperplasia usually regresses following adequate hormone replacement over a period of a few months (1). This case illustrates the importance of determining thyroid function tests during the investigation of pituitary masses (4).

References


Figure 1. Pretreatment patient – coarse facial features with large nose.

Figure 2. T1 weighted Magnetic Resonance Imaging of pituitary with gadolinium enhancement – coronal section: Pretreatment homogenous enlargement of the pituitary gland extending into suprasellar region without chiasmatic compression (A) and post treatment image with reduced size and bulkiness of the gland (B).
Pregnancy is a ‘stress test’ for the carbohydrate metabolism of all mothers-to-be. This is due to a significant increase in anti-insulin action by the placental hormones. The resulting increase of maternal insulin resistance requires a high compensatory rise of maternal insulin secretion; which when inadequate to meet the demand, manifests as gestational diabetes (GDM). Diabetes in pregnancy can be broadly divided into pre-gestational or type 2 diabetes (T2DM) and GDM. Although two distinct clinical conditions, they are a part of the continual process towards the development of T2DM and cardiovascular disease (CVD) risks in the lifecycle of a woman. Therefore GDM is now viewed as a good predictor of future diabetes in a given population. Furthermore, the offspring of mothers with poorly controlled diabetes are at greater risk of obesity and premature metabolic derangement (an inter-generational effect).

The prevalence of T2DM among our population has shown an exponential rise in the past few decades, with the age of manifestation becoming lower and with no major gender difference. The incidence of GDM has also increased exponentially, and clearly requires universal screening during pregnancy. Additionally, those identified as at high metabolic risk, require lifelong follow up beyond pregnancy and the post partum period.

Sri Lanka has a high quality primary care model for Maternal and Child Health (MCH) services under the leadership of the Family Health Bureau of the Ministry of Health. Therefore optimizing screening and management of GDM and preventing post partum progression to T2DM has implications well beyond MCH. Proper management of this “window of opportunity” for the prevention of diabetes is of extreme importance and must be viewed as a good health investment.

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance of any degree with onset or first recognition during pregnancy, irrespective of whether dietary modification alone or in combination with insulin is required (ADA 1999).

This broad definition does not distinguish between previously undetected T2DM from pregnancy related true gestational diabetes that occurs when placental hormone levels peak in mid trimester.

Screening in early pregnancy (at antenatal booking) helps distinguish between these metabolic conditions, while post partum screening both in the short term and long term would help towards a more pragmatic follow up of the mothers and families at risk.

Therefore, the Diabetes Prevention Task Force of the Sri Lanka Medical Association (SLMA), along with all stakeholders ranging from health policy makers and planners in the Ministry of Health and practicing clinicians and academics from the fields of Internal Medicine, Endocrinology, Obstetrics, Paediatrics, Community Medicine and Family Medicine identified the importance of a novel initiative called Nirogi Maatha within the Nirogi Lanka Project – phase II carried out by the SLMA. This effectively targets diabetes in pregnancy with a life cycle approach that is expected to have a health impact on a cross generational basis.

Capacity building of the MCH field staff is therefore a priority in such an endeavour. Therefore it is an essential pre-requisite to arrive at a consensus on a nationally applicable Clinical Practice Guideline for managing maternal diabetes during pregnancy. This was coordinated effectively by the Family Health Bureau in conjunction with the Sri Lanka College of Obstetricians and Gynaecologists, Ceylon College of Physicians, Endocrine Society of Sri Lanka and the Nirogi Lanka Project of the SLMA. A unique feature in this guideline is that it addresses hitherto ill addressed issues in health care viz physical activity during pregnancy, dietary options and healthy lifestyle of the mother to be and her family, as well as universal screening of pregnant women. The international divide on screening methods and cut off values for blood glucose measurement has also been effectively addressed to ensure a pragmatic approach that takes into consideration regional specific issues. Web: http://nirogilanka.org/pdf/final-gdm.pdf

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1Consultant Community Physician, National Program Manager – Maternal Care, Family Health Bureau, Ministry of Health, Sri Lanka; 2Professor in Reproductive Medicine, Consultant Physician/Endocrinologist, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Colombo, Sri Lanka.
Nirogi Maatha project –

Goal

• To improve the quality of maternal diabetes care in Sri Lanka.

• To empower communities to be responsible for the prevention of diabetes and CVD commencing from pregnancy.

Objectives

• Capacity building of field staff managing pregnant women and children specifically on issues of GDM.

• Develop nationally relevant screening method for GDM and practice guidelines in accordance with international criteria.

Target group

• Field staff in maternal and child health in the state sector through the National Coordinating Centre and Focal Point, in the Ministry of Health – the Family Health Bureau.

Intended project results

• Capacity building of human and technical resources for screening, management and long term follow up of gestational diabetes at a national level spreading out to the grass root level.

Planned activities to reach objectives

• To develop training module and train field staff on holistic management of GDM/maternal diabetes during pregnancy and beyond.

• To develop IEC material on GDM and Hypertension during pregnancy.

Expected outcomes

• Capacity building of human and technical resources for screening, management and long term follow up of gestational diabetes at a national level.

• 150 trainers, 325 Medical officers of Health (MOH), 400 Health Nursing Sisters (PHNS) field midwives (PHM) will receive appropriate training on the core subject of GDM and its importance and will be enabled to deliver the necessary health education of women of reproductive age in Sri Lanka.

• Training module available for future training of MCH staff will ensure sustainability of projects.

• The childbearing female population and families of Sri Lanka will benefit.

The cooperation and commitment to practice guidelines and their implementation by all sectors of health care in Sri Lanka will be much appreciated.
Instructions to Authors


Purpose and Scope

The Sri Lanka Journal of Diabetes Endocrinology and Metabolism (SJDEM) publishes original research articles, reviews, and other special features related to diabetes, endocrinology and metabolism in humans and human tissue.

General Information

Manuscripts must be written in fluent English and conform to the specifications described below. Manuscripts submitted to SJDEM are evaluated by peer reviewers. Authors of manuscripts requiring modifications have two (2) months to resubmit a revision of their paper. Manuscripts returned after more than two (2) months will be treated as new submissions. An unsolicited revision of a rejected manuscript will either be returned or treated as a new submission, at the editor’s discretion.

Manuscript Categories

All manuscripts must adhere to the word count limitations, as specified below, for text only; the word count does not include the abstract, references, or figure/table legends. The word count must be noted on the title page, along with the number of figures and tables.

Original Articles – Word count 3600 and include a maximum of six figures and tables and 40 references.

Brief Reports must include focused studies with important, but very straightforward, negative or confirmatory results. Word count 1800 with maximum of two figures and tables and 20 references.

Clinical Reviews and Clinical updates should address topics of importance to clinical endocrinologists, researchers and clinicians. Word count 4000 with maximum four figures and tables and 60 references. A brief section describing the search strategies used to obtain information is required for reviews.

Case Report must describe a single case or small series that reveals a novel and important insight into a condition’s pathogenesis, presentation, and/or management. The case report must include a concise scholarly review of relevant literature pertaining to the disorder. Word count 2400 with maximum four figures and tables and 20 references.

Extensive Clinical Experiences are learned descriptions of substantial clinical experience with a specific endocrine or metabolic disorder, or class of disorders, by a single clinical endocrinologist or facility. Word count 3600 and maximum four figures and tables and 40 references.

Position and Consensus Statements related to the endocrine and metabolic health standards and healthcare practices may be submitted by professional societies, task forces, and other consortia. All such submissions will be subjected to peer review, must be modifiable in response to criticisms, and will be published only if they meet the Journal’s usual editorial standards. Word count 3600 with maximum six figures and tables and 120 references.

Controversies in Clinical Endocrinology must describe and justify different approaches to diagnosis and/or management of patients with an endocrine or metabolic condition. This is ideally authored by two individuals who thoughtfully describe their respective clinical perspectives on a clinical problem, practice that must include the rationale and evidence. Word count 2400 with maximum two figures and tables and 20 references.

Images in Endocrinology require a single figure or two closely related figures that illustrate the value of visual information in clinical diagnosis of endocrine and metabolic disorders, with a caption that is 50 words or less, and a commentary of 250 words or less, and five or fewer references.

Commentaries are essentially uninvited editorials, which should concisely address and take a well-reasoned position on a timely issue of importance to clinical endocrinologists and/or investigators. Word count 1200 with maximum 10 references; no figures or tables are permitted.

Letters to the Editor may be submitted in response to work that has been published in the Journal. Letters should be short commentaries related to specific points of agreement or disagreement with the published work. Letters are not intended for presentation of original data unrelated to a published article. Word count 500 with maximum five complete references and no figures or tables.

Manuscript Submission Procedures

SJDEM only uses electronic manuscript submission. Please email to endocrinesl@gmail.com

All submissions must include:

A cover letter requesting that the manuscript be evaluated for publication in SJDEM and any information relevant to your manuscript.

Completed Copyright Assignment and Affirmation of Originality form.

Completed Disclosure of Potential Conflict of Interest form.
Manuscript Preparation

General Format

The Journal requires that all manuscripts be submitted in a single-column format that follows these guidelines:

All text should be double-spaced with 1-inch margins on both sides using 11-point type in Times Roman font.

All tables and figures must be placed after the text and must be labeled. Submitted papers must be complete, including the title page, abstract, figures, and tables. Papers submitted without all of these components will be placed on hold until the manuscript is complete.

Authors are encouraged to cite primary literature rather than review articles in order to give credit to those who have done the original work.

Any supplemental data intended for publication must meet the same criteria for originality as the data presented in the manuscript.

Title Page

The title page should include the following:

Full title (a concise statement of the article’s major contents)

Authors’ names and institutions. At least one person must be listed as an author; no group authorship without a responsible party is allowed. A group can be listed in the authorship line, but only on behalf of a person or persons. All group members not listed in the authorship line must be listed in the Acknowledgments.

Abbreviated title of not more than 40 characters for page headings

At least three key terms for indexing and information retrieval

Word count (excluding abstract, figure captions, and references)

Corresponding author’s e-mail and ground mail addresses, telephone and fax numbers

Name and address of person to whom reprint requests should be addressed

Any grants or fellowships supporting the writing of the paper

Disclosure summary (see Disclosure of Potential Conflict of Interest form for instructions)

Clinical Trial Registration Number, if applicable

Structured Abstracts

All Original Articles, Brief Reports, Clinical Reviews, Case Reports, Consensus and Position Statements, Controversies in Endocrinology, and Extensive Clinical Experiences should be submitted with structured abstracts of no more than 250 words. All information reported in the abstract must appear in the manuscript. The abstract should not include references. Write the abstract with a general medical audience in mind. Please use complete sentences for all sections of the abstract.

Introduction

The article should begin with a brief introductory statement that places the work to follow in historical perspective and explains its intent and significance.

Materials and Methods

These should be described and referenced in sufficient detail for other investigators to repeat the work.

Results and Discussion

The Results section should briefly present the experimental data in text, tables, and/or figures. The Discussion should focus on the interpretation and significance of the findings with concise objective comments that describe their relation to other work in that area.

Acknowledgments

The Acknowledgments section should include the names of those people who contributed to a study but did not meet the requirements for authorship. Each person listed in the acknowledgments must give permission for the use of his or her name.

References

References to the literature should be cited in numerical order (in parentheses) in the text and listed in the same numerical order at the end of the manuscript on a separate page or pages. Examples of the reference style that should be used are given below. For further examples refer Ann Intern Med. 1988; 108: 258-265 and Br Med J. 1988; 296: 401-405.

Journal articles and abstracts: List all authors when six or less; when seven or more, list only first three and add et al. If it is necessary to cite an abstract because it contains substantive data not published elsewhere, it must be designated at the end of the reference [e.g., 68:313 (Abstract)].

Books: List all authors or editors.

Sample References


Instructions to authors

Tables

Tables must be constructed as simply as possible and be intelligible without reference to the text. Each table must have a concise heading. A description of experimental conditions may appear together with footnotes at the foot of the table. Tables must not simply duplicate the text or figures. The width of the table must be designed to occupy one or two journal columns.

Figures and Legends

Sizing the figure: The author is responsible for providing digital art that has been properly sized, cropped, and has adequate space between images. Plan the size of the figure to fill 1, 1.5, or 2 columns in the printed journal. In most cases, figures should be prepared for 1-column width. Produce original art at the size it should appear in the printed journal. On the submission page, check boxes to indicate that the figures are the correct size and resolution.

Units of Measure

Results should be expressed in metric units. Systeme Internationale (SI units) must be added in parentheses. Temperature should be expressed in degrees Celsius (e.g., 28 C) and time of day using the 24-hour clock (e.g., 0800 h, 1500 h).

Standard Abbreviations

All nonstandard abbreviations in the text must be defined immediately after the first use of the abbreviation.

Editorial Policies and Guidelines

Prior Publication

The journal publishes original research and review material. Material previously published in whole or in part shall not be considered for publication. At the time of submission, authors must divulge in their cover letter all prior publications or postings of the material in any form of media. Abstracts or posters displayed for colleagues at scientific meetings need not be reported.

Authorship Criteria

An author should have participated in either the conception, planning, or execution of the work, the interpretation of the results and the writing of the paper. An acknowledgment accompanying the paper is appropriate recognition for others who have contributed to a lesser extent.

Experimental Subjects

To be considered, all clinical investigations described in submitted manuscripts must have been conducted in accordance with the guidelines in The Declaration of Helsinki and must have been formally approved by the appropriate institutional review committees or its equivalent. All manuscripts must indicate that IRB approval was acquired; and that when informed consent was required by the IRB, that this was obtained from subjects in experiments involving humans. Photographs of patients’ faces should be included only if scientifically relevant. Authors must obtain written consent from the patient for use of such photographs.

Experimental Animals

A statement confirming that all animal experimentation described in the submitted manuscript was conducted in accord with accepted standards of humane animal care should be included in the manuscript.

Clinical Trials Registration

Clinical trials require their prospective registration. The Clinical Trial Registration number should be provided clearly on the title page of the manuscript.

Journal Facts for SJDEM

Publisher: The Endocrine Society of Sri Lanka
Joint Editors: Dr Uditha Bulugahapitiya
Dimuthu T Muthukuda
Editorial Board: SJDEM Editorial Board
ISSN 2012-998X

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Cushing syndrome

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List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>Adreno Cortico Trophic Hormone</td>
</tr>
<tr>
<td>BIPPS</td>
<td>Bilateral Inferior Petrosal Sinus Sampling</td>
</tr>
<tr>
<td>CS</td>
<td>Cushing Syndrome</td>
</tr>
<tr>
<td>CD</td>
<td>Cushing Disease</td>
</tr>
<tr>
<td>HDDST</td>
<td>High Dose Dexamethasone Suppression Test</td>
</tr>
<tr>
<td>HPA AXIS</td>
<td>Hypothalamo Pituitary Adrenal Axis</td>
</tr>
<tr>
<td>LDDST</td>
<td>Low Dose Dexamethasone Suppression Test</td>
</tr>
<tr>
<td>ODST</td>
<td>Overnight Dexamethasone Suppression Test</td>
</tr>
<tr>
<td>UFC</td>
<td>Urine Free Cortisol</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular Filtration Rate</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin Releasing Hormone</td>
</tr>
<tr>
<td>PCOS</td>
<td>Polycystic Ovary Syndrome</td>
</tr>
<tr>
<td>TSS</td>
<td>Transphenoidal Surgery</td>
</tr>
</tbody>
</table>

Introduction

Cushing syndrome (CS) comprises symptoms and signs associated with prolonged exposure to inappropriately elevated levels of free plasma glucocorticoids. Iatrogenic CS is the most common form. Endogenous CS, may be caused by either excess ACTH secretion or independent adrenal overproduction of cortisol.

Epidemiology

Endogenous CS is a very rare entity, with an annual incidence of 2-3 cases per million individuals. The female: male ratio is 3:1. In patients whom initial cure was not obtained, a 2 to 3 fold increase in mortality is reported.

Clinical features of CS

CS often presents a diagnostic challenge, particularly in the early stages when the signs and symptoms are non-specific. As the clinical features are non-specific, presence of highly discriminative clinical features (Table 1) should prompt further biochemical tests.
## Table 1. Highly discriminative features of CS

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>% of patients</th>
<th>Discriminative index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Easy bruising</td>
<td>62%</td>
<td>10.3</td>
</tr>
<tr>
<td>Proximal muscle weakness</td>
<td>56%</td>
<td>8</td>
</tr>
<tr>
<td>Facial plethora</td>
<td>94%</td>
<td>3</td>
</tr>
<tr>
<td>Striae (esp. reddish purple and &gt;1 cm wide)</td>
<td>56%</td>
<td>2.5</td>
</tr>
</tbody>
</table>

In children: weight gain with decreasing growth velocity.

## Table 2. Less discriminative clinical features of CS

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Signs</th>
<th>Overlapping conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain</td>
<td>Dorso-cervical fat pad</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Depression</td>
<td>Facial fullness</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Obesity</td>
<td>Osteoporosis</td>
</tr>
<tr>
<td>Back pain</td>
<td>Supra clavicular fullness</td>
<td>PCOS</td>
</tr>
<tr>
<td>Decreased concentration</td>
<td>Thin skin</td>
<td>Hypokalaemia</td>
</tr>
<tr>
<td>Decreased libido</td>
<td>Peripheral oedema</td>
<td>Renal calculi</td>
</tr>
<tr>
<td>Impaired memory</td>
<td>Acne, hirsutism and female balding</td>
<td>Unusual infection</td>
</tr>
<tr>
<td>Insomnia</td>
<td>Poor skin healing</td>
<td></td>
</tr>
<tr>
<td>Irritability</td>
<td>In children – short stature</td>
<td></td>
</tr>
<tr>
<td>Menstrual abnormalities</td>
<td>Abnormal genital virilization</td>
<td></td>
</tr>
<tr>
<td>Slow growth – children</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Classification of CS

Based on etiology, CS is broadly classified into three groups as outlined in table 3.

## Table 3. Classification of CS

<table>
<thead>
<tr>
<th>ACTH Dependent</th>
<th>ACTH Independent</th>
<th>Pseudo-Cushing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cushing disease (pituitary)</td>
<td>Adrenal adenoma and carcinoma</td>
<td>Alcohol</td>
</tr>
<tr>
<td>Ectopic ACTH syndrome</td>
<td>Primary pigmented nodular adrenal hyperplasia and Carney syndrome</td>
<td>Depression</td>
</tr>
<tr>
<td>Ectopic CRH syndrome</td>
<td>McCune-Albright syndrome</td>
<td>Obesity</td>
</tr>
<tr>
<td>Macronodular adrenal hyperplasia</td>
<td>Aberrant receptor expression (gastric inhibitory polypeptide, interleukin-1α)</td>
<td></td>
</tr>
<tr>
<td>Iatrogenic (treatment with ACTH)</td>
<td>Iatrogenic (steroids)</td>
<td></td>
</tr>
</tbody>
</table>
**Diagnosis of Cushing syndrome (CS)**

After exclusion of iatrogenic CS, further investigations are recommended in following groups.

- Patients with multiple and progressive features, particularly features which are more predictive of CS
- Patients with adrenal incidentaloma
- Patients with unusual features for age (e.g. osteoporosis, hypertension)
- Children with decreasing height velocity and increasing weight (investigations for CS is not considered in obese children unless their linear growth is retarded)

**Initial investigations**

Patients with high pre-test probability should be considered for investigations. One of the four highly sensitive screening tests should be used as the initial investigation, based on the suitability for a given patient.

1. 1mg overnight dexamethasone suppression test (ODST)
2. Low dose dexamethasone suppression test (LDDST 2 mg/day for 48 h)
3. Urine free cortisol (UFC; at least two measurements)
4. Late-night salivary cortisol (two measurements)

**Table 4. Characteristics of screening tests in CS**

<table>
<thead>
<tr>
<th>Test</th>
<th>Cutoffs</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODST</td>
<td>Negative &lt;50 nmol/L (&lt;1.8 μg/dL)</td>
<td>&gt;95%</td>
<td>80%</td>
<td>See notes below</td>
</tr>
<tr>
<td>LDDST</td>
<td>Negative &lt;50 nmol/L (&lt;1.8 μg/dL)</td>
<td>&gt;96%</td>
<td>70%</td>
<td>Useful in conditions with over-activation of HPA axis. Can be followed by CRH stimulation test.</td>
</tr>
<tr>
<td>UFC</td>
<td>Assay upper limit</td>
<td>89%</td>
<td>91%</td>
<td>False positive: elevation of serum cortisol due to physiological or pathological conditions (other than CS), high fluid intake</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>False negative: renal impairment GFR &lt;60 ml/min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Check urine creatinine to verify adequacy of collection</td>
</tr>
<tr>
<td>Late night Salivary Cortisol Between 2300 h to 2400 h</td>
<td>Normal (&lt;4 nmol/L, &lt;145 ng/dL)</td>
<td>92-100%</td>
<td>93-100%</td>
<td>Easily collected at home, sample can be mailed. Not suitable for night shift workers, smokers, patients with oral ulceration, critical illness or depressive illness</td>
</tr>
</tbody>
</table>
Dexamethasone tests (ODST, LDDST): can be done as an outpatient

- Hepatic enzyme inducers (e.g. carbamazepine, phenytoin, phenobarbitone, rifampicin, alcohol) lower serum dexamethasone concentration and leads to false positive results
- Oestrogens increase CBG level leading to false positive results, needs a period off (usually six weeks) oral contraceptives before testing
- Verify patient compliance of dexamethasone during test
- For paediatric patients with weight <40kg dose should be adjusted (15 μg/kg)

Table 5. Special populations and considerations

<table>
<thead>
<tr>
<th>Special population</th>
<th>Screening consideration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td>Use UFC</td>
</tr>
<tr>
<td></td>
<td>Do not use ODST/LDDST</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>Use UFC, salivary cortisol or midnight serum cortisol</td>
</tr>
<tr>
<td></td>
<td>Do not use ODST/LDDST</td>
</tr>
<tr>
<td>Renal impairment</td>
<td>Use ODST</td>
</tr>
<tr>
<td></td>
<td>Do not use UFC</td>
</tr>
<tr>
<td>Adrenal incidentaloma</td>
<td>Use ODST</td>
</tr>
</tbody>
</table>

Special investigations

CRH stimulation test

This test is used to differentiate Cushing disease from Pseudo-Cushing syndrome. LDDST is followed by administration of CRH (1 μg/kg, IV) 2 h after the last dose of dexamethasone. Serum cortisol and ACTH are measured 15 min later. Patients with Cushing disease should respond with an increase in ACTH and cortisol. A cutoff of >207 nmol/L (>7.5 μg/dL) increases specificity to 87% but decreases the sensitivity.

Uses:
- In patients with high degree of clinical suspicion but normal UFC and negative ODST or LDDST
- Low degree of clinical suspicion but mildly elevated UFC or positive ODST or LDDST
- Patients on anti-epileptic drugs

Midnight serum cortisol test

This can be done on either sleeping or awake state. The patient should be admitted for a period of 48h or longer to avoid false-positive responses due to the stress of hospitalization. For a sleeping value, the blood sample must be drawn within 5-10 min of waking the patient, or through an indwelling line, to avoid false-positive results. A cutoff of >207 nmol/L (>7.5 μg/dL) has a sensitivity of 96% and specificity of 83%.

Uses: Easier to perform than a sleeping test but is less validated.

Sleeping midnight serum cortisol:

A single value of >50 nmol/L (>1.8 μg/dL) has a sensitivity of 100% for CS but low specificity.

Awake midnight serum cortisol:

Cutoff of >207 nmol/L (>7.5 μg/dL) has a sensitivity of 96% and specificity of 83%.

Uses: Suspect if the clinical features contrast with normal laboratory tests. Instruct the patient to collect a 24h urine sample or bedtime saliva when they feel symptoms have recurred. Repeat periodic testing when symptomatic.
Figure 1. Algorithm for initial evaluation of CS

Clinical suspicion of CS

Exclude exogenous glucocorticoid exposure

Perform one of the four screening tests

Negative test result

Positive test result

Exclude physiological causes of hypercortisolism

Refer to Endocrinologist

High probability or progressive symptoms

Perform one other screening test

Discrepant*

Suggest additional evaluation

Positive

CS

Negative

CS unlikely

* Where the patient has undergone a second test although the first screening test is negative because clinical probability of CS is very high.
Figure 2. Algorithm for diagnosing the etiology of CS

1. Biochemically/clinically confirmed CS
   - Plasma ACTH
     - Suppressed < 10 pg/mL
       - CT adrenals
       - ACTH independent disease
         - Surgery
     - Detectable > 10 pg/mL
       - ACTH independent disease
         - MRI Pituitary
         - Lesion > 6 mm
           - Proceed with surgery
         - Lesion < 6 mm or no lesion
           - BIOPSS
             - ACTH gradient > 2
               - Appropriate imaging +/− Venous sampling
               - Further evaluation

*High dose dexamethasone suppression test (HDDST): This is used to differentiate CD from ectopic ACTH. >50% suppression of plasma cortisol from the baseline value is taken as a positive response for CD. Caveat in the use of HDDST is that 90% of CD shows a positive response but 10% of ectopic ACTH also show a positive response. Up to 50% of ectopic ACTH due to bronchial carcinoids show some suppression.
Treatment and follow up

Treatment of CS involves a multi-disciplinary team approach including an endocrinologist, neurosurgeon, interventional radiologist and an oncologist. If the disease is left untreated, it carries high morbidity and mortality.

**Figure 3. Treatment of Cushing disease (CD)**

Trans sphenoidal surgery (TSS)

- Remission
- Recurrence

- Monitor for recurrence
- Recurrence
- Repeat surgery

Recurrent/persistent disease

- Radiotherapy
- Bridging medical therapy
- Bilateral adrenalectomy

**Figure 4. Treatment of Adrenal Cushing syndrome**

Adrenal Cushing syndrome

- Adrenal lesion present
  - Adrenal adenoma bilateral/unilateral
    - Surgery
  - Adrenocortical carcinoma
    - Surgery
    - Adrenolytic therapy
    - Radiotherapy

- Adrenal lesion absent
  - Further evaluation
  - Adrenal hyperplasia bilateral/unilateral
    - Further evaluation
**Surgical treatment**

**Trans-sphenoidal surgery**

The initial treatment of choice for CD is selective pituitary adenomectomy. The resection of the tumour leads to hypocortisolism as the remaining normal corticotrophs have been suppressed due to longstanding cortisol excess. The resultant hypocortisolism, in fact provides an index of surgical success.

**Peri-operative and post-operative care**

Parenteral glucocorticoids must be initiated peri-operatively (parenteral hydrocortisone 100 mg one hour prior to surgery and continued 06 hourly), and should be continued on physiological doses until the HPA axis recovers (oral hydrocortisone 12-15 mg/m² (or an equivalent) as a single morning dose or divided doses with the majority given in the morning). Postoperative hypopituitarism has to be anticipated and if detected has to be adequately replaced. During the first postoperative year, the HPA axis recovers in most patients, allowing for discontinuation of glucocorticoids. HPA axis recovery can be assessed by:

- Cortisol day curve – five measurements of serum cortisol taken at 0900, 1100, 1300, 1500, and 1700 h. A mean level of 150-300 nmol/liter (5-10 µg/dl) is equivalent to a normal production rate.
- Normalization of 24-h UFC.

**Assessment of remission**

Assessment of remission is determined by the measurement of 9am cortisol, 48 hours following surgery. Hydrocortisone must be withheld for 12 hours prior to cortisol assessment and the patient must be monitored closely for signs of hypoadrenalism. A low postoperative 9am cortisol of <50 nmol/l is associated with remission and a low recurrence rate. UFC can also be used to assess remission. Values below 55 nmol/24 h suggest remission, whereas values in the normal range of 55-276 nmol/24 h are equivocal. However, values above the normal range indicate persistent CD.

**Persistence or recurrence**

Surgical success rates are low in patients harbouring macroadenomas and in patients with tumors that have invaded the dura. While a partially resected pituitary adenoma is the commonest cause for persistent hypercortisolism, other rare possibilities such as an ectopic tumour, pseudo-Cushing and McCune-Albright syndrome has to be considered. In the event of persistence or recurrence of CD, a choice of second-line therapeutic options should be discussed with the patient.

**Adrenal surgery**

Laparoscopic unilateral adrenalectomy is the treatment of choice in adrenal adenoma.

- As the contra lateral adrenal gland is suppressed due to inhibition of ACTH, peri operative and post-operative steroid replacement is necessary.
- Assessment of remission is done by measuring 9.00 am cortisol, 48 hours after surgery as in pituitary disease. Recovery of HPA axis can be assessed by periodic monitoring of cortisol day curves.
- Following surgery, histology should be evaluated to exclude adrenocortical carcinoma.

**Medical treatment of CS**

Medical treatment of CS is useful to reduce the cortisol level before definitive surgery, while awaiting the maximum efficacy of pituitary radiotherapy and in the treatment of acute, potentially life threatening complications of CS.
Figure 5. Adrenal-blocking drugs and the site of action

Ketoconazole
- Dose: starting dose 200-400mg daily up to 800 mg daily.
- Side effects: hepatotoxicity, hypogonadism in men.
- Biochemical remission – monitor blood pressure and glycaemic regulation.

Metyrapone
- Dose: 250 mg bid to 1.5 g 6 hourly.
- Can lead to overstimulation of adrenal androgens and mineralocorticoids.
- Side effects: hirsutism, acne, hypokalemia

Mitotane
- Dose: 2 g/day in divided doses up to 10g/d if tolerated.
- Taken up by both normal and malignant adrenals. Also causes mineralocorticoid deficiency.
- Side effects: adrenal crisis, GI disturbances, neurotoxicity

Follow-up evaluation
- Evaluate for resolution of clinical features.
- Normalize of 24-h UFC.
- Cortisol day curve. (As mitotane increase cortisol binding globulin, markers of free plasma cortisol (24h UFC) is preferred).

The dosage of the above drugs can be adjusted periodically according to these parameters.
Etomidate
- Dose: i.v. 0.03 - 0.3 mg/kg/h
- Fast acting and i.v. – useful for acute/life threatening CS.
- Evaluation with serial 9am cortisol levels and serum potassium.

Pituitary-directed drugs

Pasireotide
- Dose: S.C. 600 μg bid
- Side effects: hyperglycaemia
- Response has to be monitored clinically as well as with UFC and serum cortisol.

Glucocorticoid receptor antagonists

Mifepristone
- Dose: 300 – 1200 mg/day
- Can improve glycaemia and diastolic hypertension.
- Side effects – hypokalemia, endometrial hyperplasia
- Response can be monitored by clinical parameters such as weight loss, improvement of glycaemic control and diastolic blood pressure.
- Biochemical markers such as serum cortisol and UFC cannot be used to assess the response.
Annexure

Protocol for biochemical evaluation of CS

Preparation: Patients need to be off all oestrogen containing medication for six weeks prior to any measurement of serum cortisol.

Overnight dexamethasone suppression test

Preparation: performed as an outpatient.

Protocol: advise patient to take 1mg of dexamethasone (in children 15 μg/kg body weight) at 2300 or 0000h.
Collect serum for cortisol levels at 0800 or 0900h in the following morning.

Low dose dexamethasone suppression test

Preparation: Ensure patient is not taking any steroids or oestrogen or drugs that increase the metabolism of dexamethasone (e.g. carbamazepine, phenytoin, phenobarbitone, rifampicin).

Protocol: Advise the patient to take 0.5mg of dexamethasone exactly six hourly for 2 days (0900, 1500, 2100, 0300, 0900, 1500, 2100, 0300h).
Collect serum for cortisol at 0900h after ingestion of 8 doses.

Urinary free cortisol level
Adviser the patient to collect urine for 24 hours in a plain container.
At least two measurements of urinary cortisol should be performed.

Midnight plasma and salivary cortisol levels

Saliva is collected between 2300 or 0000 h by passive drooling or by placing a cotton pledget in the mouth and chewing for 1-2 minutes.
The sample is stable at room temperature or refrigerator for several weeks.
At least two measurements of salivary cortisol should be performed.
**High dose dexamethasone suppression test**

Collect serum for a baseline 8 am cortisol measurement prior to giving dexamethasone tablets.

Advise the patient to take 2 mg dexamethasone (in children 80-120 μg/kg/day divided into four doses every 6 hours or a maximum of 2 mg every 6 hours for 2 days) every 6 hours for 2 days. (0900, 1500, 2100, 0300, 0900, 1500, 2100, 0300 h)

Collect serum for cortisol at 0800 h after ingestion of 8 doses.

Other method; advise the patient to take 8 mg dexamethasone orally at 2300 h, with measurement of an 0800 h or 0900 h cortisol level the next day.

**9 am ACTH level**

Collect serum for ACTH level at 0800 h or 0900 h.

Samples should be kept in an ice water bath, centrifuged, separated and frozen within a few hours.

Simultaneous plasma cortisol levels also should be measured.

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**References**