SCRENNING OF CUSHING'S SYNDROME IN PATIENTS WITH POORLY CONTROLLED TYPE 2 DIABETES WITH HYPERTENSION AND OBESITY.

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Introduction: Cushing’s syndrome (CS) may be unrecognized in patients with diabetes. There is no consensus on routine screening for CS in patients with type 2 diabetes (T2DM). Aim of the study was to evaluate the prevalence of unsuspected CS in out-patients with diabetes.

Methods: Cross sectional prospective study was conducted at diabetes clinic in National Hospital of Sri Lanka among patients with diabetes who were attending for out-patient visit from January-2016 to January-2017. Total of 287 patients were investigated with over-night-dexamethasone-suppression test (ODST) as screening test. Patients who fail to suppress serum cortisol less than 50nmol/l were further tested by Low-dose-dexamethasone-suppression test (LDDST). A third step midnight cortisol measurement was performed in patients who were failed to suppress cortisol less than 50nmol/l on LDDST. Fourth step imaging studies with pituitary MRI, abdominal-CT or CT-scan of chest abdomen and pelvis depending on the ACTH levels were performed.

Results: Out of 287, 46.18% (133) patients failed to suppress cortisol to less than 50nmol/l on ODST. Among these 133, 23(11.49% of total) patients failed to suppress cortisol less than 50nmol/l on LDDST. Nine out-of 23 patients had cortisol more than 140nmol/l on midnight-cortisol test, confirming true CS. Further investigations with imaging revealed one with pituitary adenoma, one with adrenal adenoma and 6 had normal imaging.

Conclusion: Considering the prevalence of definitive CS of 3.18% among poorly controlled T2DM patients with hypertension and obesity suggest that CS is not rare as previously thought. But in our Asian population with T2DM and high prevalence of co-morbidities, taking overnight dexamethasone suppression test alone as a screening test would not be suitable test to screen CS.

Introduction:-
Cushing’s syndrome (CS) reflects the biological effects of excessive endogenous or exogenous cortisol secretion. When the hypercortisolism is mild, there will be difficulties in differentiating true CS from Cushing’s phenotype and
metabolic syndrome. Although the prevalence of Cushing’s syndrome is very small in general population, it may be more prevalent in certain high risk patient populations with poorly controlled type 2 diabetes (T2DM), hypertension and obesity (Federica Guaraldi, 2012). Some studies show the prevalence of CS among patients with T2DM with poor glycemic control is much higher than in the general population (Gungunes, et al. 2014). Among the different studies there is a wide range from 0 to 9.4% in the prevalence of CS in patients with T2DM (Krarup, et al. 2012; Bogdan Catargi, et al. 2003). Among hypertensive patients the prevalence of CS is 2.1% in one study and some evidence suggests high prevalence of CS among simple obesity (Omura, et al. 2004; Sabin SB, et al. 2013).

Studies show patients with incidentally diagnosed adrenal tumors with subclinical hypercortisolism experience clinical and biochemical improvement in diabetes, hypertension and obesity after the removal of adrenal tumor (Reincke, et al., 1992). This confirms that screening for a rare disorder will be more productive if done in a well-targeted high risk cohort. Number of studies have attempted to examine the prevalence of CS in patients with T2DM though no consensus has emerged on value of routine screening for CS in T2DM. Therefore we designed this study to estimate the prevalence of CS in a cohort of overweight type 2 diabetes patients with hypertension and poor glycaemic control.

Subjects and Methodology:-
This cross sectional prospective study was conducted at diabetes clinic in National Hospital of Sri Lanka among patients with diabetes who were attending for out-patient visit from January 2016 to January 2017. Two hundred and eighty seven consecutive patients who fulfilled following inclusion criteria were included in this study: age above 18 years, poorly controlled type 2 diabetes with HbA1c of 8% or more, obesity (BMI of 27KgM$^2$ or more) and hypertension (blood pressure more than 140/80mmHg) or on antihypertensive medications. Diagnosed patients with pre-existing CS, patients with severe nephropathy with eGFR <30ml/min/M$^2$ or patients who are on medications which interfere the screening and confirmatory tests were excluded from this study.

Ethical approval and patient consent: The study was approved by the ethical committee of university of Colombo and permission from the director, National Hospital of Sri Lanka was obtained for utilizing the data and conducting the study. Informed written consent was obtained from all patients.

Materials and protocols:-
The patients in the inclusion criteria were investigated with over-night dexamethasone suppression test (ODST) as screening test for CS. Patients were advised to take 1mg dexamethasone orally at 2300h and blood samples were collected on the following morning at 0800 for determination of plasma cortisol concentration. Patients who fail to suppress serum cortisol less than 50nmol/l were further tested by Low dose dexamethasone suppression test (LDDST). For LDDST patients were advised to take 0.5mg dexamethasone orally 6 hourly for 48 hours at 0900, 1500, 2100, 0300 and serum cortisol was measured at time 0 and 48h. A third step midnight cortisol measurement was performed in patients who were failed to suppress cortisol less than 50nmol/l on LDDST as confirmatory test for presence of CS. Midnight serum cortisol was done while the patient was asleep after at least 48 hours hospitalization. A serum cortisol value higher than 140nmol/L was characterized CS. When a value was lower than 50nmol/l, it ruled out the diagnosis. Basal ACTH levels were obtained in patients with elevated midnight cortisol. All hormonal tests was carried out at chemical pathology lab, National Hospital of Sri Lanka by radio immune assay. As a fourth step imaging studies with pituitary MRI, abdominal CT or CT scan of chest abdomen and pelvis depending on the ACTH levels were performed.

Statistical analysis:-
Results are given as means with standers deviation. The comparison between variables (age, duration of diabetes, BMI, waist to hip ratio, HbA1c, blood pressure, and cortisol) among patients with CS and with-out CS were done using Pearson’s Chi-square test and Fisher’s exact test. Continuous variables were tested for normality using Shapiro Wilk test and none of the variables were normally distributed, therefore Wilcoxon sign rank test was used to compare continuous variables in CS and with-out CS groups. The level of statistical significance was considered at p<0.05. The programing language version was 3.2.3.

Results:-
The study flow chart was shown in figure 1. Total patients included to the initial screening was 287. Table 1 illustrates the baseline characteristics of the patients included. Number of patients failed to suppress cortisol to less than 50nmol/l on ODST (positive ODST) was 133 (46.18%). Further evaluation was done with LDDST for these
133 ODST positive patients. Twenty three (11.49% of total) patients failed to suppress cortisol less than 50nmol/l. A third step mid night cortisol measurement was carried out in these 23 LDDST positive patients, which revealed 9 patients with cortisol more than 140 nmol/l confirming true CS. Among these 9 patients (3.18% of total), six had basal ACTH levels more than 10pmol/l, two were in between 5 and 10 pmol/l and one had low ACTH of 5pmol/l. Further investigations revealed macroadenoma of pituitary gland on MRI in one patient. Subsequent surgery confirmed corticotroph adenoma. One patient with high ACTH declined further investigations. One had normal pituitary but 2cm right adrenal adenoma and planning for repeat scan in 6 months. No pituitary or adrenal tumour was found in five patients. One patient with ACTH of 5pmol/l underwent adrenal CT and not found any lesion. The source of the hypercortisolism was in these 6 patients remain unknown (Table 2). Table 3 shows the comparison of patients with confirmed CS (positive for all 3 screening test) and rest of patients.

Discussion:

The prevalence of CS in patients with T2DM is widely varying from 0 to 9.4% on different studies. This variation is due to number of factors including the study setting, study population and the cut-off values used for screening tests. Studies carried out among in-patients with T2DM showed prevalence of 2 to 9.4% (Leibowitz, et al. 1996; Catargi, et al. 2003; Chiodini, et al. 2005; Taniguchi, et al. 2008; Murakami, et al. 2010), whereas outpatient set-up showed prevalence of 0 to 2.9% (Caetano MS, et al. 2007; Newsome, et al. 2008; Gagliard, et al. 2010; Mullan, et al. 2010). Studies used ODST as a screening test showed more prevalence than studies used midnight salivary cortisol (2-9.4% vs 0-2.9%). The number of study population were ranged from 77 to 294 in these studies. Some studies used 140nmol/l as the cut off values for ODST and some have taken 50nmol/l. The studies carried out on newly diagnosed patients with diabetes was showed 1% of the prevalence of CS in one study and even less than 1% in another study (Reimondo, et al. 2007; Newsome, et al. 2008).

Although number of studies have attempted to examine the prevalence of CS in patients with T2DM, still no consensus has emerged on value of routine screening for CS in T2DM.

Our study set-up was out-patients with poorly controlled T2DM associated with hypertension and obesity; conferring high probability of CS. We observed nearly half of the subjects were non-suppressors for the initial screening test (ODST) indicating higher false positives in this high risk population. Two screening tests were positive in 11.49%. Only 3.18% (9 patients out of 287) was confirmed to have CS and rest of 14 were suggestive of pseudo Cushing’s based on low mid-night cortisol.

The Endocrine society guidelines currently recommends initial use of one test with high diagnostic accuracy: late night salivary cortisol, 24 hour urine free cortisol and over-night or low dose dexamethasone suppression test (Lynnette et al., 2008). Patients with abnormal result are recommended to undergo a second test with either one of the above or serum midnight cortisol or dexamethasone – CRH test. Patients with CS characteristically have loss of circadian rhythm with absence of late night cortisol nadir (Glass, et al., 1984). This forms the basis of measurement of midnight serum or late night salivary cortisol. ELISA and Liquid chromatography – mass spectrometry are the best validated methods used to measure salivary cortisol (Baid. Et al., 2007). Urinary free cortisol has high accuracy, it measures the unbound cortisol and therefore not affected by conditions and medications that alter the binding (Elamin, et al., 2008). These two methods are easily performed and less invasive tests. Non availability of these tests in our country and higher percentage of false positive results in older, diabetic or hypertensive subjects made us to choose other two recommended tests. In the dexamethasone suppression test, a lower cutoff of 50nmol/l is recommended by the experts to increase the sensitivity greater than 95%. A sleeping midnight cortisol less than 50nmol/l effectively excludes CS in a population with low clinical index suspicion (24 Pecori Giraldi, et al. 2007).

Our study protocol used testing strategy to minimize number of false positive results also more convenient and less expensive tests. However with this protocol we may have missed a number of patients with subclinical or mild hypercortisolism. Even with confirmed CS, source of the hypercortisolism was not found in the majority of the patients. The earlier reports of mortality in CS was described among individuals with severe hypercortisolism and most deaths were caused by vascular and infectious complications (Plotz, et al., 1952). Successful normalization of cortisol was resulted in reduction of the standard mortality ratio. But there are limited and conflicting data regarding whether specific treatment of patients with mild hypercortisolism is superior to medical treatment of co-morbidities alone (Reincke 2000; Terzolo, et al. 2004; Tsagarakiset al. 2006; Mitchell, et al. 2007).
Conclusions:-
In the present study nearly half of the patients exhibit lack of suppression to ODST. Therefore in our population with T2DM and high prevalence of co-morbidities, taking ODST alone as a screening test would not be suitable test to screen CS. We need at least two screening tests and preferably 3 tests to exclude pseudo Cushing’s and reduce the number of imaging tests. Biochemical abnormalities of the hypothalamic pituitary adrenal (HPA) axis on 2 screening tests were positive in 11.49%, but only 3.18% (9 patients) showed higher midnight cortisol. Whether the remaining 14 patients would have progressed towards overt CS is doubtful. The prevalence of CS among patients with T2DM is comparable to other studies, though it would be even higher if we took only 2 positive tests. Definitive diagnosis with pathological results and origin of the lesion was only found in one patient whereas 6 patients only showed biochemical evidence of CS. This could be occult CS with negative imaging or functional activation of HPA axis observed in obesity and diabetes.

Considering the prevalence of definitive CS of 3.18% among poorly controlled T2DM patients with hypertension and obesity suggest that CS is not rare than previously thought. Whether this would justify wide-scale screening is uncertain and even in this targeted population with high probability of CS, determining the cause of CS was challenging.

Declaration of Interest: there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Figure 1: Flow charts of the study

ODST: over-night dexamethasone suppression test, LDDST: low dose dexamethasone suppression test

Total patients - 287
ODST (cut-off 50nmol/l)

ODST positive patients - 133 (46.18%)
(Serum cortisol > 50nmol/l)

ODST negative patients - 154 (53.82%)
(Serum cortisol < 50nmol/l)

LDDST (cut-off 50nmol/l)

LDDST positive patients: 23 (11.49%)
(Serum cortisol > 50nmol/l)

LDDST negative patients: 110 (88.51%)
(Serum cortisol < 50nmol/l)

Midnight cortisol (cut-off 50nmol/l)

Midnight cortisol > 140nmol/l: 9 patients
Further investigations with ACTH and imaging
Pituitary adenoma - 1
Adrenal adenoma - 1
Normal imaging - 6

Midnight cortisol < 50nmol/l: 14 patients
Pseudo Cushing's Under observation

No further investigations
**Table 1:** Baseline characteristics of patients included in the study

| Variables                      | Mean | SD  |
|-------------------------------|------|-----|
| Age (years)                   | 55.7 | 8.5 |
| Duration of Disease (years)   | 13.1 | 7.5 |
| BMI (kg/m²)                   | 31.1 | 3.6 |
| Waist/hip ratio (WHR)         | 0.99 | 1.8 |
| Systolic blood pressure (SBP) (mmHg) | 137.4 | 18.6 |
| Fasting blood glucose (FBS) (mg/dl) | 153.8 | 55.4 |
| HbA1C (%)                     | 9.9  | 1.5 |
| Total cholesterol (TC) (mg/dl) | 182  | 40.5 |
| LDL cholesterol (mg/dl)       | 107.7| 34.9 |
| ALT                           | 35.0 | 18.1 |
| Total body fat %              | 38.7 | 5.5 |

**Table 2:** Characteristics of patients with confirmed Cushing’s syndrome

| Patient | Age (Y) | ODST nmol/l | LDDST nmol/l | Mid-night cortisol nmol/l | ACTH pmol/l | Imaging | Patient outcome |
|---------|---------|-------------|--------------|----------------------------|-------------|---------|-----------------|
| 1       | 49      | 68          | 65           | 245                        | 255         | MRI – 2.2x2.5cm pituitary adenoma | TSA confirmed corticotroph adenoma |
| 2       | 60      | 55          | 55           | 263                        | 30.7        | Declined further investigations | |
| 3       | 60      | 62          | 50           | 156                        | 12.2        | Normal pituitary MRI and CT Chest and abdomen | |
| 4       | 57      | 55          | 68           | 325                        | 8.3         | Normal pituitary MRI and CT Chest and abdomen | |
| 5       | 59      | 80          | 55           | 345                        | 5.1         | CT abdomen - normal | |
| 6       | 50      | 60          | 66           | 246                        | 8.6         | Normal pituitary MRI and CT Chest and abdomen | |
| 7       | 63      | 393         | 60           | 168                        | 13.7        | CT abdomen – 2cm Right adrenal mass | Repeat CT in 6 months |
| 8       | 67      | 75          | 60           | 443                        | 18          | Normal pituitary MRI and CT Chest and abdomen | |
| 9       | 57      | 56          | 61           | 254                        | 21          | Normal pituitary MRI and CT Chest and abdomen | |

ODST; overnight dexamethasone suppression test, LDDST; low-dose dexamethasone suppression test, TSA; transsphenoidal adenectomy

**Table 3:** Comparison of patients with confirmed Cushing’s syndrome (positive for all 3 screening test) and rest of patients

| Variables (median) | Patients with confirmed CS (N=9) | Patients with-out CS (N=278) | P value (Wilcoxon test) |
|-------------------|----------------------------------|------------------------------|-------------------------|
| Age (year)        | 59(56-61)                        | 56(50-62)                    | 0.40                    |
| Duration of T2DM (year) | 16(15-18)                       | 12(8-17)                     | 0.15                    |
| BMI (kg/m²)       | 30(28-34)                        | 31(28-33.8)                  | 0.98                    |
| Waist/hip ratio (WHR) | 0.9(0.9-0.9)                    | 0.9(0.8-0.9)                 | 0.19                    |
| Systolic blood pressure (SBP) (mm Hg) | 140(130-140)                    | 135(120-150)                 | 0.73                    |
| Fasting blood glucose (FBS) (mg/dl) | 167(135-171)                    | 145(112-184)                 | 0.46                    |
| HbA1c              | 11(8.4-12.1)                     | 9.6(8.8-10.8)                | 0.26                    |
| Total body fat %   | 41.3(39.2-42.9)                  | 39.4(35.6-42.4)              | 0.29                    |
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