ACCURACY OF BASELINE SERUM CORTISOL IN DIAGNOSING SECONDARY ADRENAL INSUFFICIENCY

DR NIK NUR AMIRAH BINTI NIK ABEED

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LIST OF ABBREVIATIONS

AI	Adrenal insufficiency
ACTH	Adrenocorticotropic hormone
CRH	Corticotropin releasing hormone
FN	False Negative
FP	False Positive
HSNZ	Hospital Sultanah Nor Zahirah
HRPZ	Hospital Raja Perempuan Zainab
HUSM	Hospital Universiti Sains Malaysia
HPA	Hypothalamo-pituitary-adrenal
LHR+	Likelihood Ratio Positive
LHR-	Likelihood Ratio Negative
NPV	Negative Predictive Value
PPV	Positive Predictive Value
RAAS	Renin Angiotensin-Aldosterone System
TP	True Positive
TN	True Negative
SST	Short Synacthen Test

ABSTRAK

Latar belakang: Kekurangan fungsi adrenal bermaksud adrenal korteks tidak berupaya untuk merembeskan hormon yang mencukupi seperti hormon glukokortikoid dan mineralokortikoid. Individu perlu menjalani Ujian Synacthen Pendek untuk mengenal pasti kekurangan fungsi adrenal.Untuk menghasilkan diagnosis kekurangan fungsi adrenal.Salah satu cara adalah dengan mengambil serum kortisol asas dan mengkaji tahap nilai serum tersebut .Objektif kajian ini adalah untuk membandingkan nilai serum kortisol asas dengan positif dan negatif ujian synacthen pendek yang dijalankan ke atas individu dan menilai ketepatan serum kortisol asas pada nilai yang berbeza dalam mengenalpasti kekurangan fungsi adrenal.

Kaedah: Kajian retrospektif selama 5 tahun ini dilakukan di tiga buah hospital berpakar. Seramai 111 individu yang menjalani ujian synacthen pendek di klinik endokrin telah dikenal pasti.Kekurangan funsgi adrenal primer tidak termasuk dalam kajian ini.Perbezaan kekurangan fungsi adrenal primer and sekunder bergantung kepada serum ACTH yang diambil semasa ujian synacthen test pendek dijalankan.Nilai serum ACTH yang tinggi spesifik untuk kekurang fungsi adrenal primer.Individu yang memenuhi kriteria kajian seterusnya dikenal pasti. Positive ujian synacthen pendek merujuk kepada individu yang telah disahkan diagnosis kekurangan fungsi adrenal sekundder (nilai kortisol serum <550 nmol/L pada 30 minit atau 60 minit).Serum kortisol asas didefinisikan sebagai sampel serum kortisol yang pertama diambil ketika individu menunjukkan gejala kekurangan fungsi adrenal Perkaitan antara serum kortisol asas dan tahap positif ujian synacthen pendek telah ditunjukkan oleh analisis ujian T sampel bebas. Ketepatan diagnostik dinilai oleh analisis lenkung ROC.

Keputusan: Daripada 111 individu yang menjalani ujian synacthen pendek,103 telah memenuhi kriteria yang ditetapkan. 53 (51%) telah didiagnosis mempunyai kekurangan fungsi adrenal sekunder. Lengkung ROC bagi menilai ketepatan diagnostik mempunyai kawasan di bawah lengkung 0.72 (95%CI 0.62-0.82). Untuk nilai serum kortisol asas < 80nmol/L dan <100 nmol/L dengan kekhususan tertinggi sebanyak 98%, serum kortisol yang optimum >400nmol/L mempunyai kekhususan tertinggi sebanyak 98.1%.

Kesimpulan: serum kortisol asas < 100 nmol/L menunjukkan tahap spesifik yang tinggi untuk mengenal pasti kekurangan fungsi adrenal. Faktor klinikal and bio klinikal tidak mempunyai kaitan dengan positif SST dalam kajian ini.

ABTRACT

Background: Adrenal insufficiency (AI) is defined as inability of adrenal cortex to secrete enough number of hormones likes glucocorticoids and mineralocorticoids. To establish the diagnosis of AI, one need to underwent Short Synacthen Test (SST). However, baseline serum cortisol may be a useful tool for screening and diagnosis of AI. The objectives of the study are to compare baseline serum cortisol at presentation with positive and negative SST and to evaluate diagnostic accuracy of baseline serum cortisol at different cut off value.

Methods: This 5-year retrospective study was performed in three tertiary centres. A total of 111 subjects who underwent SST in the endocrine clinics were identified. Primary adrenal insufficiency was excluded in this study. Primary and secondary adrenal insufficiency were differentiated based on serum ACTH level taken during SST. High serum ACTH suggestive of primary AI. The SST result in subjects who fulfilled the

criteria were reviewed. Positive SST refers to a patient who has confirm diagnosis of secondary adrenal insufficiency based on short synacthen test (serum cortisol level <550 nmol/L at 30 minutes or 60 minutes). Baseline serum cortisol was defined as a random serum cortisol sample drawn at first presentation. The association between random baseline serum cortisol and positive SST level were demonstrated by independent sample T test analysis. Diagnostic accuracy was evaluated by a receiver operating characteristic curve (ROC) analysis.

Result: Of the 111 patients who underwent SST, only 103 patients who fulfilled all the criteria. From 103 patients, 53 (51%) were confirmed to have secondary AI. The ROC curve for the model assessing the diagnostic accuracy had an area under curve of 0.72 (95%CI 0.62-0.82). The cut off level of baseline serum cortisol for lower group were < 80 nmol/L and < 100 nmol/L with highest specificity of 98%, the optimal baseline serum cortisol of >400nmol/L had the highest sensitivity of 98.1%.

Conclusion: Baseline serum cortisol of < 100 nmol/L is highly specific and very strongly associated with secondary Adrenal Insufficiency (AI). There is no clinical and biochemical factors associated with positive SST in this study.

Keywords:

Adrenal insufficiency, baseline serum cortisol, positive short synacthen test,

CHAPTER 1: INTRODUCTION

Adrenal insufficiency(AI) is defined as inability of adrenal cortex to secrete enough amount of hormones likes glucocorticoids and mineralocorticoids .It can be lethal if undiagnosed and left untreated . (Bornstein et al., 2016; Crowley et al., 2014). AI can be divided into two types, Primary adrenal insufficiency, and secondary adrenal insufficiency. Primary adrenal insufficiency is originated from adrenal gland itself in which it has lost its own function, for example is Addison disease. (Younes & Younes, 2017), whereas Secondary adrenal insufficiency can result from inadequate stimulation of the adrenal glands due to either insufficiency or inadequate secretion of adrenocorticotropic hormone (ACTH).

Pituitary gland is a gland situated at the base of the brain and it controls hormonal glands including adrenal glands as well as other gland such as thyroid, ovaries and testicles. ACTH known as adrenocorticotropic hormone secreted from anterior part of pituitary gland and it is important to control the secretion of cortisol hormone. The causes of secondary AI include hypothalamic defects, hypopituitarism, defects in synthesis and processing of ACTH and chronic glucocorticoid use. The commonest cause is prolonged use of glucocorticoids mainly for anti-inflammatory especially for those diagnosed with connective tissue disease(Caramori et al., 2019; Paragliola et al., 2017).

Clinical and biochemical factors of adrenal insufficiency.

There are lots of factors contributing to secondary adrenal insufficiency and play a rule in predictive of biochemical adrenal insufficiency. Features including anorexia, fatigue, gastrointestinal abnormalities, weight loss and low blood pressure are non-specific and overlap with other co-morbidities. (Ben-Shlomo et al., 2014). Age ,hypertension, clinical cushingoid appearance with history of exogenous use of steroid and those with hyponatremia are the significant clinical predictive of adrenal insufficiency (Ishikawa et al., 2001; Manosroi, Phimphilai, et al., 2020; Tarantini et al., 2007). The reported incidence of AI in inpatients is approximately 15.2/105 hospitalized people. The diagnosis of AI in inpatients is challenging due to multiple laboratory sources of interference. Normally, 20% of circulating cortisol is bound to albumin, while all the rest is bound to corticosteroid-binding globulin (CBG). Thus, the measurement of serum cortisol could be interfered by any condition that affects CBG or albumin. For instance, in patients with cirrhosis, nephrotic syndrome or severe sepsis, the levels and binding affinity of CBG to cortisol could be altered.(Chen et al., 2013; Verbeeten & Ahmet, 2018)

Diagnosis of secondary adrenal insufficiency.

To establish diagnosis of AI, one need to undergo a procedure known as short synacthen test (SST). This short synacthen test (SST) has been validated to be the most reliable tool for diagnosing AI. This test is a dynamic test and usually conducted in clinical practices due to high reliability and safety .Guideline suggests the standard dose (250g for adults) iv corticotropin stimulation (30 or 60 min) test over other existing and peak cortisol levels below 500 nmol/L (18 g/dL) at 30 or 60 minutes.(Bornstein et al., 2016). Cortisol response to ACTH is slightly higher at 60 than at 30 min, but there are no documented advantages in sensitivity

and specificity for the use of each time-point(Mayenknecht et al., 1998). A low serum cortisol that does not rise after ACTH administration (SST) confirms impaired adrenocortical reserve.(Gleeson et al., 2003)

Serum morning cortisol tests with a proper cut-off level can be used to screen for AI. Using ACTH stimulation as a reference standard for AI, some studies have proposed lower and upper cut-off values for serum morning cortisol levels of<100–145 nmol/L (3.5– 5.2 μg/dL) and >375–500 nmol/L (13.6–18.0 μg/dL), respectively.(Le Roux et al., 2002)(Perton et al., 2017). Currently, clinical practice guideline state that there is no evidence supporting the use of basal cortisol levels to diagnose AI.(Bornstein et al., 2016). However, using basal levels for screening has many advantages because it is more convenient for patients and health care providers. In fact, one study showed evidence supporting the use of basal cortisol levels to predict AI. They proposed that if the basal cortisol was >450 nmol/L (16.3 μg/dL), AI could be ruled out, with a negative predictive value of 98.7%. If the basal cortisol level was <100 nmol/L (3.6 μg/dL), +AI can be diagnosed with a positive predictive value of 93.2%(Struja et al., 2017)

The aim of this study is to compare the baseline serum cortisol level at presentation with positive and negative SST. Hence, we will be able to determine the discriminant ability and the cut off level of baseline serum cortisol in diagnosing secondary adrenal insufficiency. In addition, the study will demonstrate the association between the clinical and biochemical factors with secondary AI. There is no such study has been done in this country particularly in Kelantan and Terengganu states in which the availability of the short synacthen test is only available in these study centres. Hence this may help other healthcare providers from other centre in this state to justify the referral for the confirmatory diagnosis of AI.

CHAPTER 2: OBJECTIVE OF THE STUDY

OBJECTIVE OF THE STUDY

2.1 GENERAL OBJECTIVE

To determine the diagnostic accuracy of baseline serum cortisol and association both clinical and biochemical factors in diagnosing secondary adrenal insufficiency

2.2 SPECIFIC OBJECTIVE

1.To compare baseline serum cortisol level with positive and negative SST

2.To evaluate discriminant ability of baseline serum cortisol in diagnosing secondary adrenal insufficiency

3.To evaluate diagnostic accuracy of baseline serum cortisol at different cut off value.

4.To determine the association of clinical and biochemical factors with positive SST

CHAPTER 3: MANUSCRIPT

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TITLE: Accuracy of Baseline Serum Cortisol in Diagnosing Secondary Adrenal Insufficiency

Author: **Nik N.Amirah NA[1]**

Corresponding author**: Wan Mohamad WMI[1], MH Mohd Ali[2], Norhayati Y[3], NM Yaacob[4], TSalwani TI[5],**

1. Department of Internal Medicine, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian Kota Bharu, Kelantan, Malaysia.
2. Endocrine Unit Hospital Sultanah Nur Zahirah Kuala Terengganu Malaysia.
3. Endocrine Unit Hospital Raja Perempuan Zainab 2, Kota Bharu Kelantan Malaysia.
4. Department Of Biostatistics, School Of Medical Sciences, University Sains Malaysia, Kubang Kerian Kota Bharu, Kelantan, Malaysia.
5. Department Of Chemical Pathology, School Of Medical Sciences, University Sains Malaysia, Kubang Kerian Kota Bharu, Kelantan, Malaysia.

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3.1 ABSTRACT

Background: Adrenal insufficiency (AI) is defined as inability of adrenal cortex to secrete enough number of hormones likes glucocorticoids and mineralocorticoids. To establish the diagnosis of AI, one need to undergone Short Synacthen Test (SST). However, baseline serum cortisol may be a useful tool for screening and diagnosis of AI. The objectives of the study are to compare baseline serum cortisol with positive and negative SST and to evaluate diagnostic accuracy of baseline serum cortisol at different cut off value.

Methods: This 5-year retrospective study was performed in three tertiary centres. A total of 111 who undergone SST in the endocrine clinics were identified. Baseline serum cortisol was defined as a random serum cortisol sample drawn at first presentation. Positive SST refers to a patient who has confirm diagnosis of adrenal insufficiency based on short synacthen test (serum cortisol level <550 nmol/L at 30 minutes or 60 minutes). The association between baseline serum cortisol and positive SST level were demonstrated by independent sample T test analysis. Diagnostic accuracy was evaluated by ROC analysis.

Result: Of the 111 patients who underwent SST, only 103 patients who fulfilled all the criteria. From 103 patients, 53 (51%) were confirmed to have secondary AI. Mean serum cortisol for positive SST was 143.86 ± 105.68 nmol/L.The ROC curve for the model assessing the diagnostic accuracy had an area under curve of 0.72 (95%CI 0.62-0.82). The cut off level of baseline serum cortisol for lower group were < 80 nmol/L and < 100 nmol/L with highest specificity of 98%, the optimal baseline serum cortisol of >400nmol/L had the highest sensitivity of 98.1%.

Conclusion: Baseline serum cortisol of < 100 nmol/L is highly specific and very strongly associated with secondary Adrenal Insufficiency (AI). There were no clinical and biochemical factors associated with positive SST in this study.

Keywords:

Adrenal insufficiency, baseline serum cortisol, positive short synacthen test,

3.2 INTRODUCTION

Adrenal insufficiency (AI) is defined as inability of adrenal cortex to secrete enough amount of hormones likes glucocorticoids and mineralocorticoids .It can be lethal if undiagnosed and left untreated (1,2). AI can be divided into two types, primary adrenal insufficiency, and secondary adrenal insufficiency. There are factors contributing to secondary adrenal insufficiency such as age of the patient ,presence of hypertension, clinical cushingoid appearance with history of exogenous use of steroid(3). Clinical presentation including anorexia, fatigue, gastrointestinal abnormalities, weight loss and low blood pressure are non-specific symptoms to predict AI. For screening of AI, serum morning cortisol tests with a proper cut-off level can be used to screen AI. There is no evidence to support the use of basal cortisol levels to diagnose AI in clinical practice guideline(1). However, using baseline levels for screening has many advantages because it is more convenient for patients and health care providers. Patients need to undergo short synacthen test (SST) which has been validated to be the most reliable tool for diagnosing AI. This test is a dynamic test and usually conducted in a clinical practice due to high reliability yet not invasive.

The aim of this study is to compare the baseline serum cortisol level with positive and negative SST. Hence, we will be able to determine the discriminant ability and the cut off level of baseline serum cortisol in diagnosing secondary adrenal insufficiency. In addition, the study will demonstrate the association between the clinical and biochemical factors with secondary AI. There is no such study has been done in this country particularly in Kelantan and Terengganu states in which the availability of the short synacthen test is only available in these study centres. Hence this may help other healthcare providers from other centre in this state to justify the referral for the confirmatory diagnosis of AI.

3.3 METHODOLOGY

Study Population and Study Design

This is a cross sectional study on diagnostic accuracy of baseline serum cortisol in diagnosing secondary adrenal insufficiency. The study population involved all those patients who underwent short synacthen test (SST) in 3 tertiary care medical centres (HUSM Kubang Kerian, HRPZ 2 Kota Bharu and HSNZ, Kuala Terengganu) under follow up outpatient endocrine clinic from 1st June 2016 to 1st June 2021.

The SST result of the patients who underwent the test were reviewed. The result of the patients who fulfilled the criteria were included in the study. The inclusion criteria were those aged more than 18 years old and diagnosed as adrenal insufficiency. The exclusion criteria were patients who diagnosed with primary adrenal insufficiency based on SST. Primary adrenal insufficiency was diagnosed when the serum ACTH level taken during SST was high. Women who took oral contraceptive therapy and those who were hospitalised and critically ill were excluded. Patients who had undergone surgical intervention within 2 months also were excluded in this study.

The SST results of patients who fulfilled the criteria were further divided into positive and negative SST. Positive SST refers to a patient who has confirm diagnosis of secondary adrenal insufficiency based on short synacthen test (serum cortisol level <500 nmol/L at 30 minutes or 60 minutes). Baseline serum cortisol was defined as a random serum cortisol sample drawn at first presentation. The association between random baseline serum cortisol and positive SST level were demonstrated by independent sample T test analysis. Diagnostic accuracy was evaluated by a receiver operating characteristic curve (ROC) analysis.

Short Synacthen Test

Short Synacthen Test is a dynamic test conducted mainly for confirmatory diagnosis of adrenal insufficiency. Prior to procedure, patient receiving glucocorticoid were instructed to stop taking those medication 24 hours before testing. At 0-minute serum cortisol and serum ACTH were measured followed by intravenous administration of 250 mcg Tetracosactrin (ACTH analogue). Serum cortisol at 30 minutes and 60 minutes following administration of intravenous Tetrosactrin were measured. Normal response of the test when the serum cortisol level at 30 minutes or 60 minutes are more than 550nmol/L.

Data Collection

The demographics, underlying history and clinical data for patients were retrieved from medical record of patients visited for short synacthen test at adult endocrinology clinics in 3 tertiary hospitals

Definitions

In this study, random baseline serum cortisol was defined as a serum cortisol sample drawn at first presentation or before starting steroid replacement therapy. Short synacthen test (SST) was defined as a test involved in measuring serum total cortisol (nmol/L) at 0-minute,30 minutes, 60 minutes, and serum ACTH at 0 minute following intravenous administration of 250 mcg tetracosactrin (ACTH analogue). Positive SST refers to who has confirmed diagnosis of secondary adrenal insufficiency based on short synacthen test with low or normal serum ACTH

and serum cortisol level <500 nmol/L at 30 minutes or 60 minutes. Primary AI defined as when the SST result showed high serum ACTH and serum cortisol level < 500 nmol/L at 30 minutes or 60 minutes. Normal ACTH defined as cut of point-less than 10 pmol/L.

Statistical Analysis

All statistical analysis were performed with IBM Statistic Program for Social Sciences (SPSS version 26) software. Categorical variables are presented as frequency and percentage whereas numerical variables are presented as mean and standard deviation for normally distributed variables. For non-normally distributed numerical variables, medians and interquartile ranges are presented. To compare baseline serum cortisol and positive SST, we analysed using an independent sample T test. The statistical significance level was set as P value <0.05. The areas under the ROC curves (AUC) of the model were plotted to evaluate the discriminant ability of baseline serum cortisol to differentiate with adrenal insufficiency and non-adrenal insufficiency and to determine the diagnostic accuracy of each cut off value. Cut of value of baseline serum cortisol were grouping into lower and upper cut off values based on the sensitivity and specificity and optimal cut off value were calculated using Youden index. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio of positive (LHR+), likelihood ratio of negative (LHR -) and AUC for cut off values were reported.

The association of clinical and biochemical factors with positive diagnosis of adrenal insufficiency were analysed using logistic regression and were reported as crude regression coefficient with a 95% confidence interval (CI).

Sample Size Calculation

Sample size calculation for association of serum baseline cortisol with positive SST and negative SST is calculated using G power 3.1 software

Power: 80% alpha 5%

Minimum sample size needed:110

Sample size calculation for association of chemical and biochemical factors with positive SST determined by using PS software using T test (2 independent means)

Minimum sample size needed:142

Based on study by: (4)

Ethical issue

Ethical approval was obtained from Jawatankuasa Etika Penyelidikan (Manusia) – JEPeM, Universiti Sains Malaysia on 10th November 2021 with study protocol code USM/JEPeM/21090606 and National Medical Research Register (NMRR) Malaysia on 8th February 2022 with NMRR ID-21-02417-AUS

3.4 RESULTS

Patient Sociodemographic and Clinical Characteristics

Overall study flow is shown in figure 1. Total number of patients with suspected adrenal insufficiency underwent short synacthen test (SST) were 111 patients. Eight patients were excluded from this study including those with primary adrenal insufficiency, aged less than 18 years old and incomplete data of SST result.

Of all 111 patients, a total of 103 patients were fulfilled the inclusion criteria. The demographic, clinical, biochemical factors and short synacthen test results were reviewed. SST results were divided into negative and positive SST. From 103 patients, 51% (n=53/103) had positive SST and were diagnosed as secondary adrenal insufficiency. The mean age of patients in positive SST group were older compared to negative SST group (55.32 ± 16.29) (43.90 ± 17.17). Proportion of male patients is higher in positive SST group (n:26) compared to negative SST group (n:17), whereas female is predominantly higher in negative SST (n:33) compared to positive SST (27). For systolic and diastolic blood pressure, the result was almost similar for both groups and the p value were not significant. For comorbidities, majority of the patients had hypertension and diabetes mellitus type 2 in both groups. In positive SST group ,57.1% of them were smokers compare to negative SST group 42.9% of patients were smoker. Exogenous steroid is the main cause of secondary AI in positive SST group (56.2%). However, about 43.8% of patient had history of taking exogenous steroid in negative SST group. Those who diagnosed with positive SST, most of them significantly presented with fatigue compared to negative SST group (p value<0.001). Other symptoms observed in this study were not significant. About 63.8% in positive SST group were started steroid replacement therapy prior to SST (p-value:0.025). The median results of biochemical investigation in both groups shown

no significant differences, however serum ACTH in the positive SST group showed slightly lower compared to negative SST group with p value of 0.667.

Comparison Of Baseline Serum Cortisol with Positive And Negative SST

The mean baseline serum cortisol at presentation demonstrated positive SST was 143.86 ± 105.68 nmol/L however mean baseline serum cortisol demonstrated negative SST was 216.48 ± 87.18 nmol/L. (Figure 2, Table 2). It showed baseline serum cortisol in positive SST is lower compared to baseline serum cortisol in negative SST.

Diagnostic Accuracy and Cut Off Level Baseline Serum Cortisol in Diagnosing of Secondary AI.

The baseline serum cortisol in the study showed a higher diagnostic accuracy in the diagnosing of secondary AI. The ROC curve for the model assessing the diagnostic accuracy had an area under curve of 0.72 (95%CI 0.62-0.82). (Figure 3) (Table 3)

For cut off level of baseline serum cortisol, the level further divided into two groups lower and upper-level groups (Table 4). In lower level group of baseline serum cortisol, the cut off level of baseline serum cortisol for diagnosing secondary AI was < 100 nmol/L with specificity of 98% ,PPV was 95.7% and the LHR+ was 20.8. For the upper-level group of baseline serum cortisol, the optimal baseline serum cortisol of >400nmol/L had the highest sensitivity of 98.1% and specificity of 4.0%.

Association Of Clinical and Biochemical Factors in Positive SST

There were no significant association between systolic blood pressure, diastolic blood pressure and heart rate with positive SST (p value >0.05) in terms of clinical factors. For biochemical factors there were also no significant association between serum glucose, serum albumin, serum potassium and serum sodium with positive SST (p value >0.05) (Table 5).

3.5 DISCUSSION

In this study, majority of the patient who had AI were elderly compared to no AI with statistically significant of p value < 0.05 and for the gender, the proportion of female and male who diagnosed AI were similar. This is consistent with the previous study in which most of the patient diagnosed AI aged more than 50 years old and the percentage of the male and female were the same(5,6). In our study, symptom of fatiguability demonstrated to be statistically significant clinical features of secondary AI with p value of less than 0.05 .This findings showed similarity with other studies done showing the incidence of nausea, vomiting and fatigue are the major symptoms in AI(7–10). A previous study also reported that AI can occur in 6.8%- 60% of patient with exogenous steroids (11).Similarly in our study ,more than half of patients documented to have AI had history of taking exogenous steroid. Steroid is the most common drug that can cause suppression of hypothalamic pituitary axis hence resulted in drug induced adrenal insufficiency.

Mean serum cortisol for positive SST was 143.86 ± 105.68 nmol/L. The result was lower compared to previous study, which reported mean serum morning cortisol was 216.22 nmol/ L and mean basal serum cortisol was 171.58 nmol/L (4). Discriminant ability of baseline serum cortisol based on AUC – 0.72 (0.62- 0.82). The result comparable with Manosroi et al 2019 (6), serum morning cortisol AUC:0.82 (0.75-0.88) and basal serum cortisol AUC: 0.69 (0.62-0.78).

We highlighted that certain baseline serum cortisol level at presentation regardless of the time has statistically significance for diagnostic performance in establishing a diagnosis of secondary adrenal insufficiency. The findings are comparable with latest study that showed the performance of basal serum is better than serum morning cortisol level(1,12).

SST is a diagnostic tool for AI for the clinician to decide on initiating the treatment however in a certain centre which SST is not available and need immediate treatment, the baseline serum cortisol may help to guide the clinician for diagnosing AI. This also help in reducing the cost and the need of specific cut off level of baseline serum cortisol will lead a clinician to justify the need of further workup and treatment. This study showed that the level of serum cortisol of <100 nmol/L has higher specificity of 98% and sensitivity of 41.5%. This study is consistent with the previous study from Debono *et al* which showed the lower cut off level of basal serum cortisol was also < 100nmol/L (10,12). In contrast with the previous study, the cut off value for basal cortisol level was reported < 85nmol/L (6).The upper cut off level for baseline serum cortisol with high sensitivity may also help the clinician in ruling out AI in a real clinical practice. Our proposed upper cut off level for baseline serum cortisol was > 400 nmol/L with high sensitivity of 98.1%. In contrast with the previous study by Struja T *et al* reported the cut

off level was > 450 nmol/L which was slightly higher compare to our study (12). Kumar *et al* study was done previously in looking for adrenal reserve and the level of basal serum cortisol >350nmol/L was reported to predict the normal adrenal function with the sensitivity of 100% (13). The differences in the lower and upper cut off level baseline serum cortisol in our current study compare with the previous studies reported are probably contributed by the variability in the serum cortisol assay performed and the sampling time of the cortisol level. Furthermore, the cause of AI was different as in the previous study, the lower cut off baseline serum cortisol was mainly use for both primary and secondary AI (12).The diversity of population and type of steroids may influence the difference in the cut off level of basal serum cortisol for diagnosing AI.

In this study, we identified that there were no association between the clinical factors with the diagnosis of secondary AI. The findings were in concordance with the previous study.(3,5) Studies have shown that there were association between the biochemical factors such as serum sodium with positive SST(3,14) However in our study, there were no significant association between biochemical factors with positive SST. This may be explained by the effectiveness of the Renin-angiotensin-Aldosterone system (RAAS) that controlling all the mineralocorticoid activity hence all the serum biochemical results were in a normal levels and the clinical blood pressure normalized.

3.6. CONCLUSION, STUDY LIMITATION AND RECOMMENDATIONS

In conclusion, baseline serum cortisol of < 100 nmol/L is highly specific and very strongly associated with secondary Adrenal Insufficiency (AI). There were no clinical and biochemical

factors associated with positive SST in this study. This data would be beneficial to healthcare practice and clinician who are in area where SST is not readily available, to identify patient with low baseline serum cortisol, to start initial treatment first if indicated and to refer them to tertiary centre for further testing.

We acknowledge several limitations in this study. First is a small sample size study. Therefore, a larger sample size may be needed and give a better result. Second is the retrospective nature of our study. Thus, our result needs to be validated in a prospective study.

3.7 REFERENCES

1. Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, et al. Diagnosis and treatment of primary adrenal insufficiency: An endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2016;101(2):364–89.
2. Crowley RK, Argese N, Tomlinson JW, Stewart PM. Central hypoadrenalism. J Clin Endocrinol Metab. 2014;99(11):4027–36.
3. Manosroi W, Phimphilai M, Khorana J, Atthakomol P, Pipanmekaporn T. Predictive factors of adrenal insufficiency in outpatients with indeterminate serum cortisol levels: A retrospective study. Med. 2020;56(1):1–12.
4. Manosroi W, Kosachunhanan N, Atthakomol P. Clinical and biochemical factors to predict biochemical adrenal insufficiency in hospitalized patients with indeterminate cortisol levels: A retrospective study. BMC Endocr Disord. 2020;20(1):1–8.
5. Manosroi W, Pipanmekaporn T, Khorana J, Atthakomol P, Phimphilai M. A Predictive Risk Score to Diagnose Adrenal Insufficiency in Outpatients: A 7 Year Retrospective Cohort Study. Medicines. 2021;8(3):13.
6. Manosroi W, Phimphilai M, Khorana J, Atthakomol P. Diagnostic performance of basal cortisol level at 0900-1300h in adrenal insufficiency. PLoS One [Internet].

2019;14(11):1–11. Available from: http://dx.doi.org/10.1371/journal.pone.0225255

7. Bancos I, Hahner S, Tomlinson J, Arlt W. Diagnosis and management of adrenal insufficiency. Lancet Diabetes Endocrinol. 2015;3(3):216–26.

8. Erichsen MM, Løvås K, Skinningsrud B, Wolff AB, Undlien DE, Svartberg J, et al. Clinical, immunological, and genetic features of autoimmune primary adrenal insufficiency: Observations from a Norwegian registry. J Clin Endocrinol Metab. 2009;94(12):4882–90.

9. Mitchell AL, Pearce SHS. Autoimmune Addison disease: Pathophysiology and genetic complexity. Nat Rev Endocrinol [Internet]. 2012;8(5):306–16. Available from: http://dx.doi.org/10.1038/nrendo.2011.245

10. Debono M, Ghobadi C, Rostami-Hodjegan A, Huatan H, Campbell MJ, Newell-Price J, et al. Modified-release hydrocortisone to provide circadian cortisol profiles. J Clin Endocrinol Metab. 2009;94(5):1548–54.

11. Broersen LHA, Pereira AM, Jørgensen JOL, Dekkers OM. Adrenal insufficiency in corticosteroids use: Systematic review and meta-analysis. J Clin Endocrinol Metab. 2015;100(6):2171–80.

12. Struja T, Briner L, Meier A, Kutz A, Mundwiler E, Huber A, et al. Diagnostic accuracy of basal cortisol level to predict adrenal insufficiency in cosyntropin testing: Results from an observational cohort study with 804 patients. Endocr Pract. 2017;23(8):949–61.

13. Kumar R, Carr P, Wassif W. Diagnostic performance of morning serum cortisol as an alternative to short synacthen test for the assessment of adrenal reserve; A retrospective study. Postgrad Med J. 2020;1–6.

14. Perton FT, Mijnhout GS, Kollen BJ, Rondeel JMM, Franken AAM, Groeneveld PHP. Validation of the 1 µg short synacthen test: An assessment of morning cortisol cut-off values and other predictors. Neth J Med. 2017;75(1):14–20.

3.8 FIGURES AND TABLE

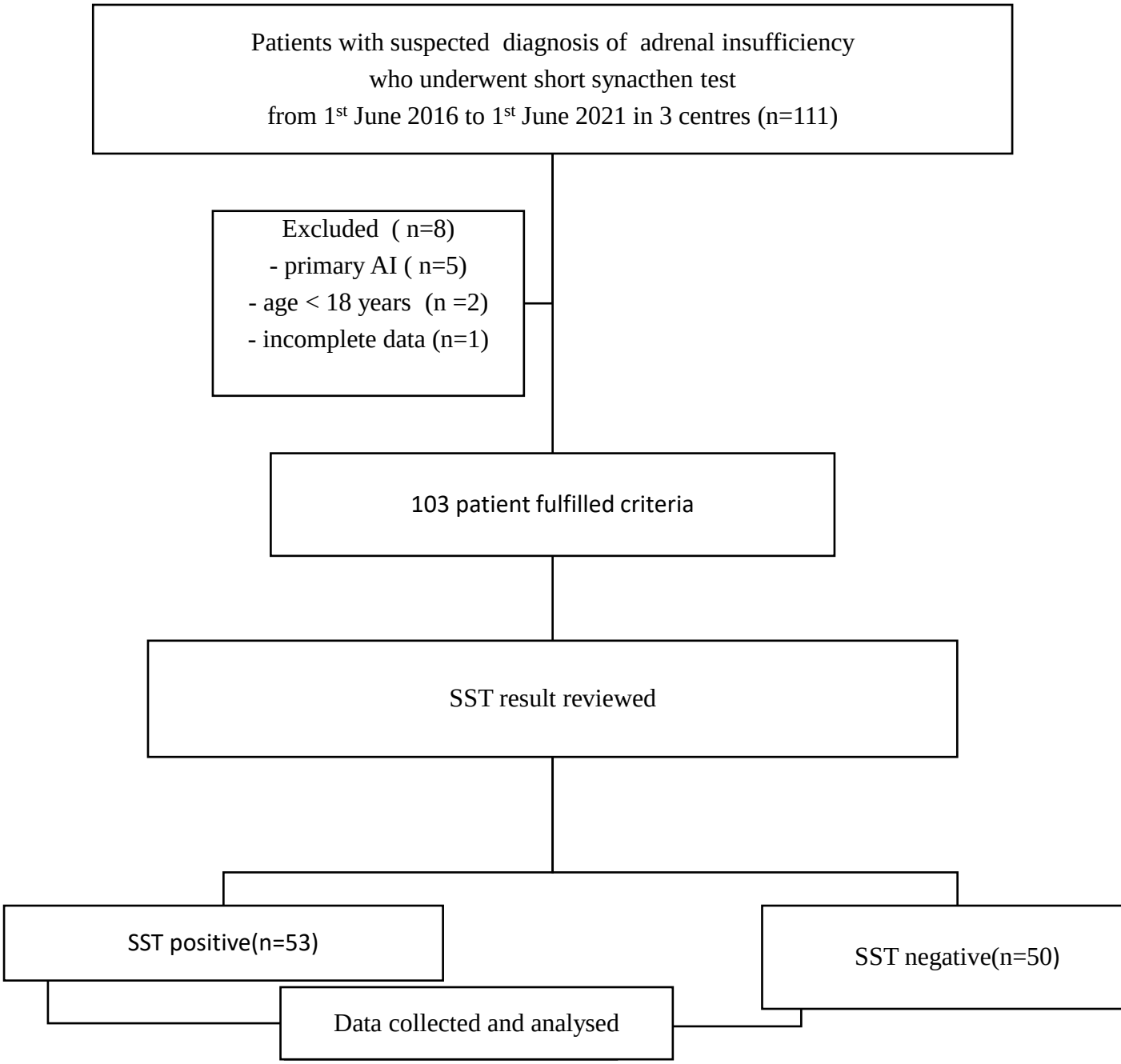

Figure 1: Study Flow

Table 1: Demographic details, clinical and biochemical characteristics for Positive and negative SST

Characteristic	SST positive (n=53)	SST negative (n=50)	P-Value
Age (mean ±SD)	55.32 ± 16.29	43.90 ± 17.17	0.001
Gender n(%)			
Male	26 (60.5)	17 (39.5)	0.162
Female	27 (45.0)	33 (55.0)	
BMI (mean ± SD) (kg/m2)	32.92 ± 5.41	31.45 ± 5.21	0.163
BP at baseline (mean ± SD) (mmHg)			
SBP	133.45 ± 23.38	129.28 ± 21.84	0.352
DBP	80.17 ± 12.70	79.36 ± 14.09	0.760
Comorbidities n (%)			
Hypertension	16 (37.2)	27(62.8)	0.720
Type 2 DM	22(59.5)	15(40.5)	0.304
Heart Disease	7(87.5)	1(12.5)	0.061
Asthma	2(66.7)	1(33.3)	1.00
Autoimmune	2(28.6)	5(71.4)	0.260
Smoking n (%)	12(57.1)	9(42.9)	0.629
Indication for SST n (%)			*0.193*
Pituitary	12(40.0)	18(60.0)	
Exogenous Steroid	41(56.2)	32(43.8)	
Symptoms At Presentation n (%)			
Fatigue	27(81.8)	6(18.2)	<0.001
Weight Lost	4(57.1)	3(42.9)	1.000
Giddiness	5(45.5)	6(54.5)	0.756
Nausea & Vomiting	3(50.0)	3(50.0)	1.000
Electrolyte imbalance	14(60.9)	9(39.1)	0.350
Hypotension	6(75.0)	2(25.0)	0.271
Hypoglycemia	5(45.5)	6(54.5)	0.756
Steroid replacement before SST n (%)	37(63.8)	21(36.2)	0.025
Biochemical Results (median ± IR)			
Glucose	5.65 ± 2.0	5.90 ± 2.6	0.396
Albumin	38.00 ± 5.5	40.00 ± 9.3	0.071
Potasium	3.90 ± 0.9	4.10 ± 0.6	0.602
Sodium	138.00 ± 5.5	136.00 ± 4.5	0.374
Serum ACTH	3.80 ± 4.4	4.13 ± 3.02	0.667

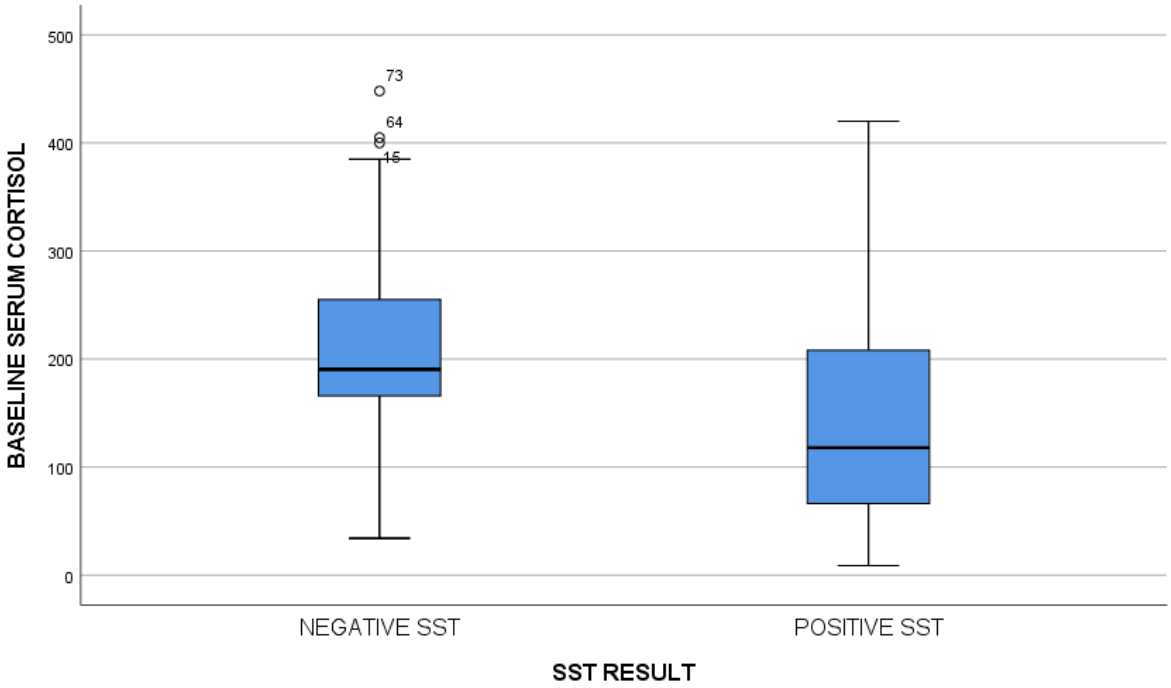

Figure 2: Box plot graph of baseline serum cortisol after SST categorized positive SST and negative SST

Table 2: Mean ± SD of baseline serum cortisol in subjects with positive and negative SST.

	SST positive (n = 53)	SST negative(n = 50)	P value
Baseline serum cortisol (nmol/L) (mean ± SD)	143.86 ± 105.68	216.48 ± 87.18	<0.001

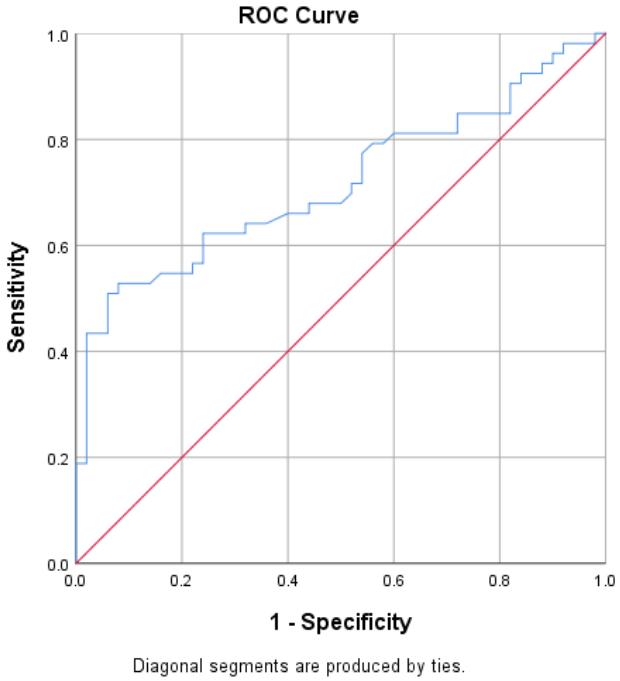

Figure 3. Area under curve of for baseline serum cortisol in secondary adrenal insufficiency.

Table 3.AUC table for baseline serum cortisol in secondary adrenal insufficiency.

	AUC	95 % CI	p-value
Baseline serum cortisol (nmol/L)	0.72	0.62 – 0.82	<0.001

Table 4: Accuracy of cut off level baseline serum cortisol in subject with positive SST.

Lower Baseline Serum Cortisol											
Baseline serum Cortisol (nmol/L)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (%)	NPV (%)	LHR+	LHR-	TP (n)	FN (n)	FP (n)	TN (n)	AUC (95%CI
<80	30.2(18.3-44.3)	98.0(89.4-99.9)	94.1	57.0	15.9	0.7	16	37	1	49	0.64(0.53-0.75)
<100	41.5(28.1-55.9)	98.0(89.4-99.9)	95.7	61.3	20.8	0.6	22	31	1	49	0.70(0.59-0.80)
<125	52.8(38.6-66.7)	92.0(80.8-97.8)	87.5	64.8	6.6	0.5	28	25	4	46	0.72(0.63-0.82)
<150	56.6(42.3-70.2)	78(64.0-88.5)	73.2	62.9	2.6	0.5	30	23	11	39	0.67(0.57-0.78)
<175	64.2(49.8-79.6)	68(53.3-80.5)	68.0	64.2	2.0	0.5	34	19	16	34	0.66(0.56-0.77)
<200	73.6(59.7-84.7)	46.0(31.8-60.7)	59.1	62.2	1.36	0.8	39	14	27	23	0.60(0.49-0.71)
Upper Baseline Serum Cortisol											
Baseline serum Cortisol (nmol/L)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (%)	NPV (%)	LHR+	LHR-	TP (n)	FN (n)	FP (n)	TN (n)	AUC (95%CI
>200	71.7(57.7-83.2)	46.0(31.8-60.7)	58.5	60.5	1.3	0.6	38	15	27	23	0.59(0.48-0.70)
>250	84.9(72.1-93.3)	26.0(14.6-40.3)	54.9	61.9	1.2	0.6	45	8	37	13	0.56(0.44-0.67)
>300	90.6(79.3-96.8)	18.0(8.58-31.4)	53.9	64.3	1.1	0.5	48	5	41	9	0.54(0.43-0.66)
>350	94.3(84.3-98.8)	12.0(4.5-24.3)	53.2	66.7	1.1	0.5	50	3	44	6	0.53(0.42-0.64)
>400	98.1(89.9-99.9)	4.0(0.4-13.7)	52.0	66.7	1.0	0.5	52	1	48	2	0.51(0.39-0.62)