Original Article

# A Comparison of Lower Doses of Porcine Sequence Corticotropin with Standard Dose in Testing the Hypothalamic Pituitary Adrenal Axis in Healthy Individuals

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## Abstract

**Context:** Porcine sequence corticotropin (PSC) stimulation test (PSCST) is a reliable, cost-effective alternative to the short Synacthen test. Long-acting PSC is widely available as a 300 IU multidose vial (60 IU per 1 ml). **Aims:** To compare the efficacy of lower doses of PSC that can be given directly from the multidose vial without reconstitution, with standard dose in assessing the hypothalamic pituitary adrenal (HPA) axis in healthy individuals. **Settings and Design:** Prospective study comparing different doses of PSC. **Methods and Material:** In 13 healthy volunteers, serum Cortisol was estimated at 30 and 60 minutes after intramuscular administration of 24IU/250 µg standard dose (0.4 ml) and lower doses of PSC (18 IU/0.3 ml/;12 IU/0.2 ml; and 6 IU/0.1 ml), with a gap of 4 weeks between each dose. **Statistical Analysis Used:** Mean ± SD was used to express quantitative variables. ANOVA and paired T-test were used for statistical analysis. **Results:** The mean ± SD of peak Cortisol levels after PSCST with all doses of PSC were >18 ug/dl. The means of peak Cortisol responses to different doses of PSC among subjects were comparable. In a subject, there was no significant dose effect and interaction (dose x time) effect indicating that the different doses were comparable (both at 30 and 60 minutes) ($p = 0.735$). **Conclusions:** All tested lower doses of PSC obtained from the multidose vial without reconstitution, including the lowest dose (6 IU/62.5 µg) tested, were comparable in efficacy to the standard dose (24IU/250 µg) in assessing the adequacy of HPA axis in healthy individuals.

**Keywords:** Acton Prolongatum, ACTH stimulation test, HPA axis suppression, low-dose porcine sequence corticotropin stimulation test, low-dose Synacthen test, porcine sequence corticotropin, Synacthen

## Introduction

Cortisol insufficiency (CI) is a common, life-threatening endocrine illness.[1,2] Insulin tolerance test (ITT) and short Synacthen test or high-dose Synacthen test (HDST) using 250 µg Cosyntropin, a synthetic ACTH (1-24) are the standard tests for hypothalamic pituitary adrenal (HPA) axis evaluation.[1-4] However, risk of dreaded complications due to hypoglycemia with ITT,[5,6] and the high cost and non-availability of Synacthen in many countries, has restricted their use. Porcine sequence corticotropin (PSC) stimulation test (PSCST) utilizes PSC, which is readily available in India as a 300 IU multidose vial (60 IU/1 ml). At a standard dose of 24 IU/ 250 µg (0.4ml), PSCST has shown efficacy comparable to 250 µg HDST and ITT in the evaluation of HPA axis in healthy individuals[7] and in patients with CI.[8-11] Both HDST and PSCST use supraphysiological dose of corticotropin, which can overstimulate adrenal gland and under diagnose mild cases of secondary CI. These cases can be picked up with the 1 µg low-dose Synacthen test (LDST),[12] which has diagnostic accuracy to comparable to ITT and HDST.[1,13-18] However, the 1 µg LDST requires the dilution of 250 µg ampule of Synacthen which is cumbersome and could result

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in low reproducibility due to dosing errors. There are no studies on efficacy of lower doses of PSC for the evaluation of HPA axis. In this study, we intend to assess the efficacy of the lowest possible doses of PSC that can be utilized without reconstitution of the commercially available preparation in healthy individuals.

## Subjects and Methods

The study was conducted at a tertiary care centre in India. Ethical clearance was obtained from the internal Ethics Committee at our institute. The participants were thirteen healthy volunteers (doctors and nurses) recruited from our hospital between the age group of 18–59 years. Those with pregnancy were excluded.

### Porcine sequence corticotropin stimulation test

The long-acting PSC is available as a 5 ml multidose vial (60 IU/1 ml) marketed as Acton Prolongatum by Ferring pharmaceuticals. The synthetic PSC in Acton Prolongatum is reversibly bound to carboxymethyl cellulose which protects against enzymatic breakdown of PSC and prolongs its action for 15 hours.[19] It has been shown that the stimulatory action of 25 IU/250 µg of PSC is equivalent to 250 µg (25IU) of Synacthen.[20] However, on calculating accurately, 250 µg of PSC corresponds to 24 IU.[7,11] We chose to compare standard dose of PSU, i.e., 24 IU/250 µg (0.4 ml) with 3 other lower doses including, 18 IU/187.5 µg (0.3 ml), 12 IU/125 µg (0.2 ml) and 6 IU/62.5 µg (0.1 ml), which could be drawn directly from the vial without reconstitution. We chose to test the above lower doses based on the ease of administration. Dose lower than 6 IU (0.1 ml) would require dilution of available vial, which would be cumbersome and could lead to dosing errors.

After an informed consent, baseline morning (8:00–9:00 AM) serum Cortisol sample was drawn in all participants [Figure 1]. Following this, PSCST was done with 24 IU/250 µg (0.4 ml) of PSC. The required dose of PSC was drawn in a 2 ml syringe and was administered intramuscularly at the deltoid site. Subsequently, the test was repeated with lower dose of PSC including 18 IU/187.5 µg (0.3 ml), 12 IU/125 µg (0.2 ml) and 6 IU/62.5 µg (0.1 ml), with a washout period of at least 4 weeks between each injection. Venous blood samples were collected at 30 minutes and 60 minutes after every injection to assess serum Cortisol response. A serum Cortisol level at 30 or 60 minutes after PSCST of more than 18 µg/dl was considered as an adequate response in the immunoassay used for the study.

The PSCST was done on Outpatient Department (OPD) basis. In all participants, we monitored vitals twice at 30 and 60 minutes after each injection. Before each subsequent PSCST after 1 month and at follow-up after 1 year, we enquired for any hypersensitivity reaction in the participants. Each multidose PSC vial was refrigerated at 2- to 4-degree Celsius. Once opened, the refrigerated PSC vial was used for a period of one month, after which it was disposed.

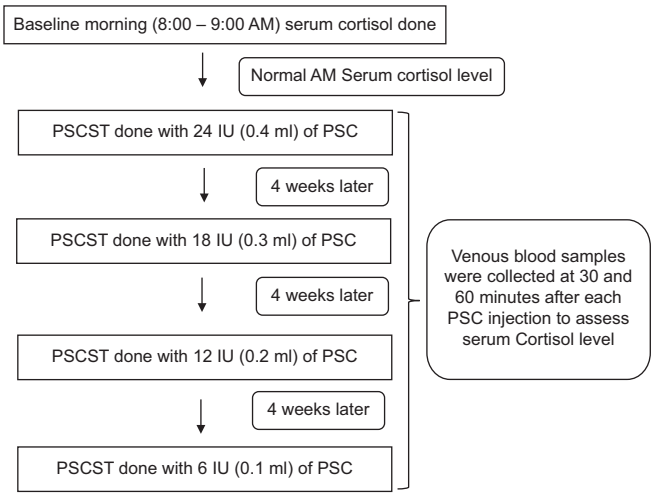


**Figure 1:** Flowchart of methodology. PSCST—porcine sequence corticotropin stimulation test. PSC—porcine sequence corticotropin

### Laboratory methods

The blood sample was collected by venepuncture in a golden yellow-capped SST (serum separator and clot activator) tube and was allowed to clot completely at room temperature for 15–30 minutes. It was then centrifuged at 3000 rpm for 10 minutes. 50 µL of serum was pipetted to the analyser. Serum Cortisol was measured using chemiluminescent microparticle immunoassay (CMIA) with Abbott Architect instrument and Architect Cortisol assay. The lower limit of detection of serum Cortisol was 0.8 µg/dl. The calibration range for the assay was 0–59.8 µg/dl. The inter-assay coefficient of variance (CV) was 1.8%, and the intra-assay CV was—Level I - 3.3%, Level II – 3.4%, Level III – 1.8%. Here, the Levels I, II, III are quality control (QC) materials (with varying concentration of analyte) used to monitor the precision of immunoassay for the analyte.

### Statistical methods and data analysis

The sample size was calculated to be 13 individuals, considering a mean difference (in the Cortisol response between groups tested with different doses of PSCST) of 4.3, a standard deviation (in the Cortisol response between groups tested with different doses of PSCST) of 2.7 (group 1) and 4.9 (group 2), and an equivalence margin of 5 units, with a power of 80% and alpha error of 5%.[20] Mean ± SD was used to express continuous variables. A paired T-test was used to compare Cortisol response to different doses of PSC among different subjects. The stimulated Cortisol values with different doses of PSCST in a subject were compared with each other using repeated measures of ANOVA. A *P* value less than 0.05 was considered significant. The Statistical Package for the Social Sciences (SPSS) software version 24.0 was used for data analysis.

### Ethical Clearance Statement

The study was approved by Institutional Ethical Committee (IEC), St John's Medical College and Hospital, Bengaluru vide IEC Study Reference number 85/2019 on 10th June 2019.

Written informed consent was obtained for participation in the study and use of the patient data for research and educational purposes.The procedures follows the guidelines laid down in Declaration of Helsinki (2013).

## Results

Thirteen healthy participants were included in the study. Among them, three were males and ten were females. The mean age of the participants was 28.5 ± 4.67 years. The mean baseline morning serum Cortisol was 11.48 ± 4.25 µg/dl. All the participants attained a peak serum Cortisol value of >18 µg/dl at either 30 or 60 minutes of administration of different doses of PSC. The mean ± SD of maximum stimulated serum Cortisol levels after 24, 18, 12 and 6 IU of PSCST were 23.9 ± 2.8, 23.7 ± 3.6, 23.3 ± 2.5, 24.1 ± 3.5 µg/dl, respectively. [Table 1] [Figure 2]

The means of peak serum Cortisol responses to different doses of PSC among participants were comparable ((24 Vs 18 IU, $P = 0.822$; 24 Vs 12 IU, $P = 0.455$; 24 Vs 6 IU, $P = 0.842$; 18 Vs 12 IU, $P = 0.652$; 18 Vs 6 IU, $P = 0.745$; 12 Vs 6 IU, $P = 0.292$)) [Table 2]. Each participant's peak Cortisol responses to different doses and time in PSCST was compared. There was no significant dose effect and interaction (dose x time) effect indicating that the different doses were comparable (both at 30 and 60 minutes). (p = 0.735). [Figure 3]

It was noted that the serum Cortisol response at 60 minutes after PSCST was significantly higher than the response at 30 minutes with all the doses of PSC ($p \leq 0.01$). However, the dose did not have a significant effect on the time to maximal response after PSCST (p = 0.631). It was noted that the serum Cortisol response at 60 minutes after PSCST was significantly higher than the response at 30 minutes with all the doses of PSC ($p \leq 0.01$) [Figure 3]. There were no reported adverse reactions to PSC administration during the study.

**Table 1: The mean±SD of baseline and peak serum Cortisol levels with 24, 18, 12 and 6 IU of porcine sequence corticotropin (PSC) administration. *N*- number of participants**

| Peak serum Cortisol levels (µg /dl) with different doses of PSC | | | | | |
|---|---|---|---|---|---|
| **PSC Dose in units** | ***n*** | **Minimum** | **Maximum** | **Mean** | **Standard deviation** |
| 0 (baseline) | 13 | 5.70 | 19.70 | 11.4846 | 4.25810 |
| 24 | 13 | 19.60 | 28.00 | 23.9154 | 2.84308 |
| 18 | 13 | 18.60 | 32.50 | 23.7538 | 3.66552 |
| 12 | 13 | 18.70 | 26.60 | 23.3077 | 2.52173 |
| 6 | 13 | 19.10 | 31.30 | 24.1077 | 3.52667 |

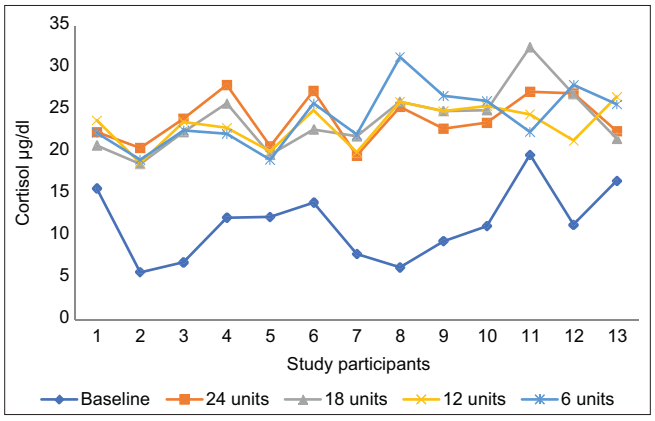


**Figure 2:** The comparison of baseline and peak Cortisol levels with different doses (24, 18, 12 and 6 units/IU) of porcine sequence corticotropin (PSC) among the 13 participants

## Discussion

The short Synacthen test or HDST with 250 µg of corticotropin is a standard test for the evaluation of HPA axis.[1,2] The short Synacthen test uses supraphysiological dose of corticotropin which overstimulates the adrenal gland, resulting in underdiagnosis of a mild form or recent onset secondary CI. These cases of mild or recent onset secondary CI can be picked up by the 1 µg LDST.[12,21] Studies have shown that LDST has comparable diagnostic accuracy to ITT and HDST or is even more sensitive than that HDST.[1,13-18]

After 250 µg of Synacthen, the concentration of corticotropin in plasma exceeds 1,00,000 pg/ml. Whereas after 1 µg Synacthen, the concentration of corticotropin in plasma exceeds 1,000 pg/ml. A plasma corticotropin concentration of >100 pg/ml induces maximum production of Cortisol by the adrenals, after which the dose-response curve flattens out.[22] Hence, dose as low as 1 µg Synacthen are more than sufficient for dynamic test for evaluation of CI. However, the 1 µg LDST requires the dilution of 250 µg ampule of Synacthen which is cumbersome and could lead to dosing errors. Non-availability and higher cost further restrict its use in many countries. The PSCST using long-acting PSC has been shown to be a reliable, cost-effective alternative to short Synacthen test and ITT in the

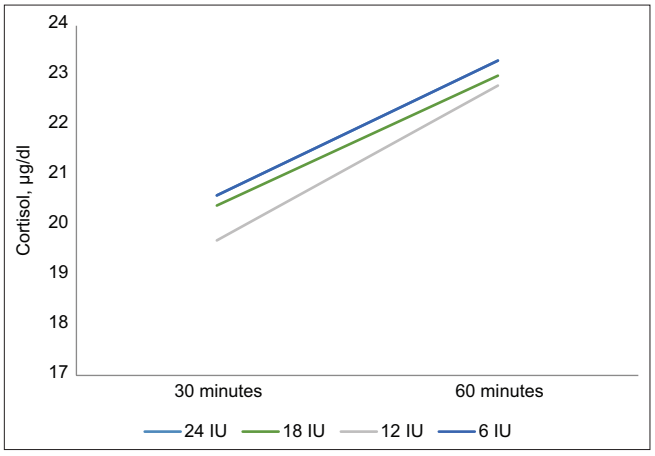


**Figure 3:** Comparison of the serum Cortisol responses at 30 and 60 minutes with different doses of porcine sequence corticotropin among all participants

**Table 2: Comparison of means of peak serum Cortisol with different doses of porcine sequence corticotropin (between doses differences). Pair 1- 24 Vs 18 IU; pair 2- 24 Vs 12 IU; pair 3- 24 Vs 6 IU; pair 4- 18 Vs 12 IU; pair 5- 18 Vs 6 IU; pair 6- 12 Vs 6 IU. ****P*** **value from paired *t*-test**

| Comparison of means of peak Cortisol with different doses of porcine sequence corticotropin (between doses differences) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Pair | Paired differences | | | | | *t* | df | *P* value sig. (2-tailed)* |
| | Mean | Standard deviation | Standard error mean | 95% confidence interval of the difference | | | | |
| | | | | Lower | Upper | | | |
| 1 | 0.16154 | 2.52670 | 0.70078 | −1.36533 | 1.68841 | 0.231 | 12 | 0.822 |
| 2 | 0.60769 | 2.83739 | 0.78695 | −1.10692 | 2.32231 | 0.772 | 12 | 0.455 |
| 3 | −0.19231 | 3.40599 | 0.94465 | −2.25053 | 1.86591 | −0.204 | 12 | 0.842 |
| 4 | 0.44615 | 3.47410 | 0.96354 | −1.65322 | 2.54553 | 0.463 | 12 | 0.652 |
| 5 | −0.35385 | 3.83463 | 1.06353 | −2.67109 | 1.96340 | −0.333 | 12 | 0.745 |
| 6 | −0.80000 | 2.61566 | 0.72545 | −2.38063 | 0.78063 | −1.103 | 12 | 0.292 |

evaluation of HPA axis in several studies done across various centres.[7-11]

However, to the best of our knowledge, efficacy of lower doses of PSC in the evaluation of HPA axis has not been assessed. Long-acting PSC is available as a 5 ml multidose vial (60 IU in 1 ml) and hence dose as low as 0.1 ml (6 IU/62.5 µg) can be administered without further dilution. In this study, we assessed the efficacy of lower doses of long-acting PSC that can be given directly from the multidose vial without reconstitution. We compared the stimulatory action of 18 IU/187.5 µg (0.3 ml), 12 IU/125 µg (0.2 ml), and 6 IU/62.5 µg (0.1 ml) PSC, with standard the dose 24 IU/250 µg (0.4 ml) in testing HPA axis in 13 healthy volunteers.

All participants had serum Cortisol values >18 µg/dl at either 30 or 60 minutes after different doses of PSC. The Cortisol response at 60 minutes after PSCST was significantly higher than that at 30 minutes with all the doses of PSC ($p = < 0.01$). The dose used in PSCST did not have a significant effect on the time to maximal response ($p = 0.631$). It can therefore be concluded that PSCST at 60 minutes can adequately test HPA axis in healthy individuals at all tested lower dose of PSC. The means of peak Cortisol responses to different doses of PSC among participants were comparable. There was no significant dose effect and interaction (dose x time) effect indicating that the different doses were comparable (both in 30 and 60 minutes). ($p = 0.735$). Thus, all lower doses tested (6,8,12 IU) in PSCST were able to adequately stimulate the HPA axis in healthy individuals comparable to the standard dose (24 IU).

Use of the lowest tested dose of PSC of 6 IU/62.5 µg (0.1 ml) instead of the standard 24 IU/250 µg (0.4 ml) would decrease the cost of investigation in each subject to one-fourth. This would be economically beneficial to the patients. A single multidose vial containing 300 IU/5 ml was previously being used for testing 12 patients with a standard dose of 24 IU/250 µg (0.4 ml). With the lowest tested dose of 6 IU/62.5 µg (0.1 ml), single vial can now be used in 50 patients. This would be of benefit in a high-volume centre.

Previous studies using PSC have looked for adverse events only in the immediate period after its administration.[7-11] No adverse reactions like anaphylaxis or delayed hypersensitivity reaction were seen among any of our participants following the four different doses of PSCST, immediately after injection or on follow-up at 1 year. In previous studies, when Synacthen was reconstituted with saline as 1 µg corticotropin and refrigerated at four degrees Celsius in plastic tubes, it was stable for four months.[14] There is spare data of the efficacy of multidose vial PSC with storage and reuse.[12] In our study, the PSC used from the stored multidose vial produced an adequate Cortisol response at all doses in all our study participants, indicating stability for the storage period of one month.

In this study, we have shown that the lowest tested doses of PSC (6 IU) obtained from multidose vial without dilution were able to adequately stimulate the HPA axis in healthy individuals comparable to the standard dose (24 IU). Further studies are required to validate and determine the diagnostic accuracy of low-dose PSCST in individuals with CI. Due to ease in administration, the lowest dose of PSC used in our study was 6 IU/62.5 µg (0.1 ml). The lowest effective dose of PSC formulated by reconstitution is yet to be ascertained.

## Conclusion

All tested lower doses of PSC obtained from the multidose vial without reconstitution, including the lowest dose (6 IU/62.5 µg) tested, were comparable in safety and efficacy to the standard dose (24 IU/250 µg) PSCST in assessing the adequacy of HPA axis response in healthy individuals.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hamme GD, *et al*. Diagnosis and treatment of primary adrenal insufficiency: An endocrine society clinical practice guideline. J Clin

Endocrinol Metab 2016;101:364–89.
2. Newell Price DC J, Auchus RJ. The Adrenal Cortex. In: Melmed S, Auchus RJ, Goldfine AB, Koenig RJ, Rosen CJ, editors. Williams textbook of endocrinology. 14th ed. Copyright © by Elsevier; 2020; p. 480-541.
3. Crowley RK, Argese N, Tomlinson JW, Stewart PM. Central hypoadrenalism. J Clin Endocrinol Metab 2014;99:4027-36.
4. Jones SL, Trainer PJ, Perry L, Wass JA, Bessser GM, Grossman A. An audit of the insulin tolerance test in adult subjects in an acute investigation unit over one year. Clin Endocrinol (Oxf) 1994;41:123-8.
5. Mahajan T, Lightman LS. A simple test for growth hormone deficiency in adults. J Clin Endocrinol Metab 2000;85:143-7.
6. Petersenn S, Quabbe HJ, Schofl C, Stalla GK, von Werder K, Buchfelder M. The rational use of pituitary stimulation tests. Dtsch Arztebl Int 2010;107:437-43.
7. Wagmode NB, Mathew V, Bantwal G, Ayyar V, George B, Patanjali CP, *et al*. It is time to Carpe Diem with porcine ACTH-A comparison of porcine sequence corticotropin to tetracosactide hexaacetate in testing the hypothalamic pituitary adrenal axis in healthy individuals. Indian J Endocrinol Metab 2018;22:636-40.
8. Gundgurthi A, Garg MK, Dutta MK, Pakhetra R. Intramuscular ACTH stimulation test for assessment of adrenal function. J Assoc Physicians India 2013;61:320–4.
9. Sharma R, Madathil S, Maheshwari V, Roy K, Kumar N, Jain V. Long-acting intramuscular ACTH stimulation test for the diagnosis of secondary adrenal insufficiency in children. J Pediatr Endocrinol Metabol 2019;32:57-63.
10. Nair A, Jayakumari C, George GS, Jabbar PK, Das DV, Jessy SJ, *et al*. Long-acting porcine sequence ACTH in the diagnosis of adrenal insufficiency. Eur J Endocrinol 2019;181:639–45.
11. Atluri S, Sarathi V, Goel A, Channabasappa S, Alapaty S, Dhananjaya MS, *et al*. Long-acting porcine sequence ACTH (Acton Prolongatum) stimulation test is a reliable alternative test as compared to the gold standard insulin tolerance test for the diagnosis of adrenal insufficiency. Indian J Endocr Metab 2022;26:38-43.
12. Park YJ, Park KS, Kim JH, Shin CS, Kim SY, Lee HK. Reproducibility of the cortisol response to stimulation with the low dose (1 microg) of ACTH. Clin Endocrino (Oxf) 1999;51:153–8.
13. Ospina NS, Al Nofal A, Bancos I, Javed A, Benkhadra K, Kapoor E, *et al*. ACTH Stimulation tests for the diagnosis of adrenal insufficiency: Systematic review and meta-analysis. J Clin Endocrinol Metab 2016;101:427–34.
14. Dickstein G, Shechner C, Nicholson WE, Rosner I, Shen-Orr Z, Adawi F, *et al*. Adrenocorticotropin stimulation test: Effects of basal cortisol level, time of day, and suggested new sensitive low dose test. J Clin Endocrinol Metab 1991;72:773–8.
15. Gonzalbez J, Villabona C, Ramon J, Navarro MA, Gimenez O, Ricart W, *et al*. Establishment of reference values for standard dose short synacthen test (250 microgram), low dose short synacthen test (1 microgram) and insulin tolerance test for assessment of the hypothalamo-pituitary-adrenal axis in normal subjects. Clin Endocrinol 2000;53:199–204.
16. Abdu TA, Elhadd TA, Neary R, Clayton RN. Comparison of the low dose short synacthen test (1 microg), the conventional dose short synacthen test (250 microg), and the insulin tolerance test for assessment of the hypothalamo-pituitary-adrenal axis in patients with pituitary disease. J Clin Endocrinol Metab 1999;84:838–43.
17. Mak IYF, Au Yeung BYT, Ng YW, Choi CH, Iu HYP, Shek CC, *et al*. Salivary cortisol and cortisone after low-dose Corticotropin stimulation in the diagnosis of adrenal insufficiency. J Endocr Soc 2017;1:96–108.
18. Kosak M, Duskovai M, Starkai L, Jandikova H, Pospisilova H, Sramkova M, *et al*. Can the gold standard be beaten? How reliable are various modifications of the synacthen test compared to the insulin tolerance test. Physiol Res 2017;66:S387-95.
19. Acton Prolongatum (Corticotrophin Carboxymethylcellulose Injection BPC'73) [Package insert]. Thane, India: Ferring Pharmaceuticals Private Limited; February 2023.
20. Upton GV, Dorothy R, Hollingsworth, Lande S, Lerner A, Amatruda T. Comparison of purified human and porcine ACTH in man. J Clin Endocrinol Metab 1970;30:190-5.
21. He X, Findling JW, Auchus RJ. Diagnosis and Management of Adrenal Insufficiency. In: Levine AC, editor. Adrenal Disorders. Physiology, Pathophysiology and Treatment. In Poretsky L, series editor. Contemporary Endocrinology. © Springer International Publishing AG; 2018, p. 199-216.
22. Findling JW. Ten rules of Adrenal Insufficiency. In: 2019 Meet- the-Professor. Endocrine case management. Copyright © by Endocrine Society; 2019. p. 32-6.