

## SALIVARY CORTISOL LEVELS AS A PROGNOSTIC MARKER IN PATIENTS WITH ACUTE SUBARACHNOID HEMORRHAGE

Dimitar MONOV, Nikolai LILYANOV

<sup>1</sup>. Department of Anesthesiology and Intensive Care, University Hospital "St. Ivan Rilski" Sofia, Bulgaria, Medical University Sofia.

---

### Abstract

#### Introduction

The stress response to aneurysmal subarachnoid hemorrhage (aSAH) has not only a beneficial effect and may become an additional cause of vasospasm. The stress response is mediated by the action of endogenous production of corticosteroids and catecholamines. The measurements of blood cortisol levels, which have been the most widely used to assess the stress response, are characterized by certain limitations. The aim of our study was to establish the relationship between the level of cortisol measured in saliva in patients with aSAH, the level of Glasgow Coma Scale (GCS) and the rate of mortality in the department of the intensive care unit (ICU).

#### Material and Methods

Our study included 78 patients with aSAH treated with endovascular embolization for cerebral aneurysm with daily examination of the level of cortisol in saliva at 08.00 AM and at 08.00 PM until ICU discharge or death. Daily salivary cortisol levels, hemodynamic parameters (mean arterial pressure), neurological status and level of consciousness (GCS) were monitored.

#### Results

Our study included 35 women and 43 men. The mean age of the patients was 64.2 years (from 42 years to 77 years). Eighteen patients died (14.06%). Salivary cortisol levels were significantly higher in deceased patients, both in the morning (31,8 nmol/l for deceased patients vs. 16,8 nmol/l for patients discharged alive;  $p < 0.001$ ) and in the evening during the follow-up period (5,2 nmol/l for deceased patients vs. 3,6 nmol/l for patients discharged alive;  $p < 0.001$ ). We found a statistically significant correlation between the increase in the morning salivary cortisol level and the decrease of the score of GCS in 11 out of 18 deceased patients. The same correlation for the evening salivary cortisol was found in only 5 patients.

#### Conclusions

The measurement of salivary cortisol in patients with aSAH provides additional information about the prognosis. Patients who will die during the acute phase of aSAH have higher morning and evening salivary cortisol compared to patients who will survive. Patients who will die have demonstrated a progressive increase of the morning salivary cortisol.

---

### Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) has an annual incidence of 5-6 per 100 000 person years. The 30-day mortality rate is 20-25% and did not change significantly during the last years. The most common cause of death and disability following aSAH is cerebral ischemia from cerebral vasospasm (1, 2). The stress response to aSAH has not only a beneficial effect and may become an additional cause of vasospasm. The stress response is mediated by the action of endogenous production of corticosteroids and catecholamines. The measurement of blood cortisol levels, which have been the most widely used to assess the stress response, is characterized by certain limitations. The process of taking blood samples from the vein is accompanied by additional stress, which results in falsely positive results. Another disadvantage is the fact that cortisol taken and measured from serum or plasma represents total cortisol, not the free, biologically active cortisol. The measurement of morning and evening salivary cortisol may monitor the free cortisol levels in the serum and may be an effective and convenient screening test for stress response (3). Elevated levels of cortisol in saliva are associated with a higher stress-induced response in patients with SAH and lower GCS score during treatment in ICU(4) The aim of our study was to establish the relationship between the level of cortisol measured in saliva in patients with aSAH, the level of Glasgow Coma Scale

(GCS) and the rate of mortality in the department of the intensive care unit (ICU).

## **Materials and Methods**

### **Study design:**

This was a prospective observational cohort study of patients with aSAH who was treated between January 2015 and September 2022 in the Intensive Care Unit (ICU) of the Department of Anesthesiology and Intensive Care at “St. Ivan Rilski” Hospital ,Sofia. The trial was approved by the Ethical committee of University Hospital “St. Ivan Rilski”.

### **Inclusion and exclusion criteria:**

Our study included patients with aSAH treated with endovascular embolization for cerebral aneurysm with daily examination of the level of cortisol in saliva at 08.00 AM and at 08.00 PM until ICU discharge or death. Saliva samples were collected from each patient using Salivette (Sarstedt). Salivary ELISA Kit was used for the quantitative measurement of cortisol in saliva samples. Seventy eight consecutive patients were treated for aSAH with endovascular embolization between January 2015 and September 2022. Endovascular embolization was performed within 24 hours from the admission of the patient. Sixteen patients were excluded because of problem with the numbers or timing of saliva collection. Finally, 78 patients were included in our study. All patients with subarachnoid hemorrhage were treated in the intensive care unit (ICU) according to the same treatment protocol:

- 1.Nimodipine i.v
- 2.Prophylactic hypertension.
- 3.Vasopressor-induced elevation of mean arterial pressure.
4. Normal blood oxygenation and saturation.
5. Maintain MAP around or above 100mmHg.

### **Observed parameters:**

We monitored daily salivary cortisol levels, hemodynamic parameters (mean arterial pressure), neurological status and level of consciousness (GCS). Data were collected until ICU discharge (average 12.2days, from 7 days to 14 days) or death.

### **Statistical analysis:**

Statistical analyses were completed using SPSS (version 22, IBM Corp., Armonk, NY, USA). A descriptive analysis of the consecutive time points measurements of mean salivary cortisol levels, Glasgow coma scale, and mean arterial pressure was performed. Data are presented as mean  $\pm$  standard deviation (SD). The associations between the mean salivary cortisol levels and the Glasgow coma scale were analyzed using Pearson’s correlation coefficient  $r$ . A  $p$ -value below 0.05 was considered statistically significant.

## **Results**

Our study included 35 women and 43 men. The mean age of the patients was 64.2 years (from 42 years to 77 years). Eighteen patients died (23%).

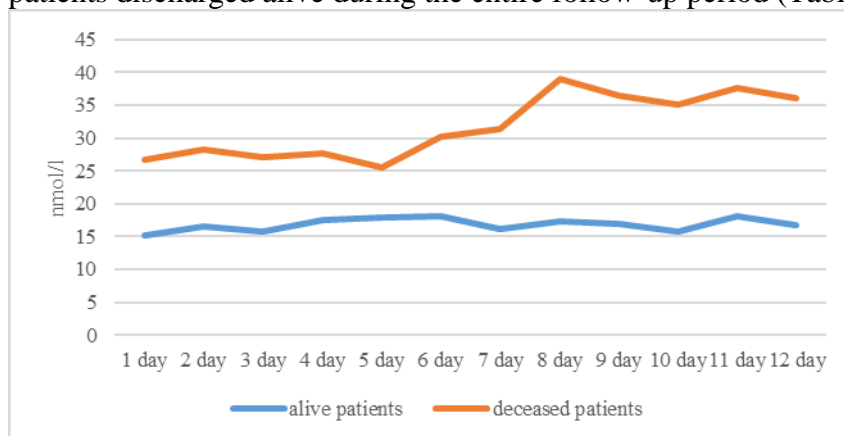
Salivary cortisol levels were significantly higher in deceased patients, both in the morning (31,8 nmol/l for deceased patients vs. 16,8 nmol/l for patients discharged alive;  $p < 0.001$ ) and in the evening during the

follow-up period (5,2 nmol/l for deceased patients vs. 3,6 nmol/l for patients discharged alive;  $p < 0.001$ ). There was no difference regarding mean arterial pressure (table 1).

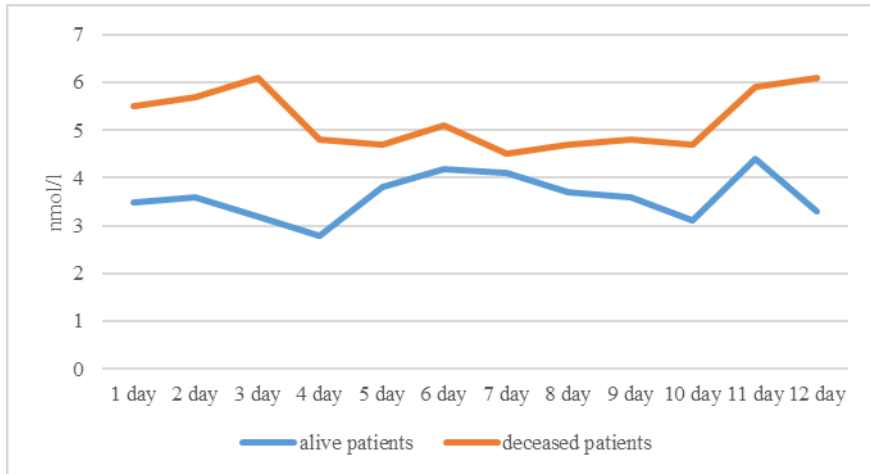
**Table 1.** Mean salivary cortisol levels (nmol/l) at 8h. and 20 h. and mean arterial pressure (mmHg) among alive and deceased patients

day	Discharged alive patients			deceased patients		
	mean salivary cortisol levels nmol/l - 8h.	mean salivary cortisol levels nmol/l - 20 h.	MAP - mean arterial pressure mmHg	mean salivary cortisol levels nmol/l - 8h.	mean salivary cortisol levels nmol/l - 20 h.	MAP - mean arterial pressure mmHg
1 day	15,2	3,5	89	26,7	5,5	91
2 day	16,5	3,6	88,0	28,2	5,7	92
3 day	15,7	3,2	91,0	27,1	6,1	88
4 day	17,5	2,8	85,0	27,7	4,8	87
5 day	17,9	3,8	93,0	25,5	4,7	85
6 day	18,1	4,2	94,0	30,3	5,1	89
7 day	16,2	4,1	84,0	31,4	4,5	94
8 day	17,3	3,7	87,0	39,1	4,7	91
9 day	16,9	3,6	88,0	36,5	4,8	90
10 day	15,8	3,1	88,0	35,1	4,7	92
11 day	18,1	4,4	92,0	37,7	5,9	91
12 day	16,7	3,3	94,0	36	6,1	87
mean values	16,8	3,6	89,4	31,8	5,2	89,8

Salivary cortisol levels have remained significantly higher in the morning and in the evening in deceased patients compared to patients discharged alive during the entire follow-up period (Table 1, Fig. 1, Fig. 2).

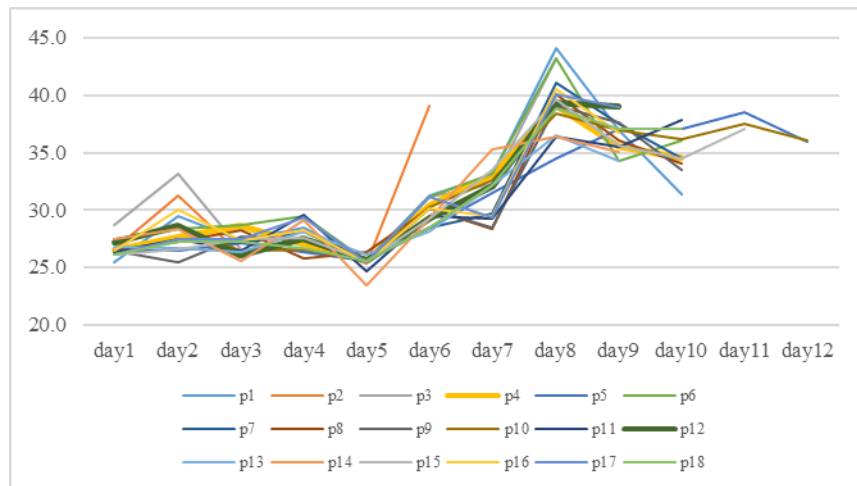


**Figure 1.** Mean salivary cortisol levels (nmol/l) among alive and deceased patients at 08:00h. in the morning ( $p < 0.001$ )

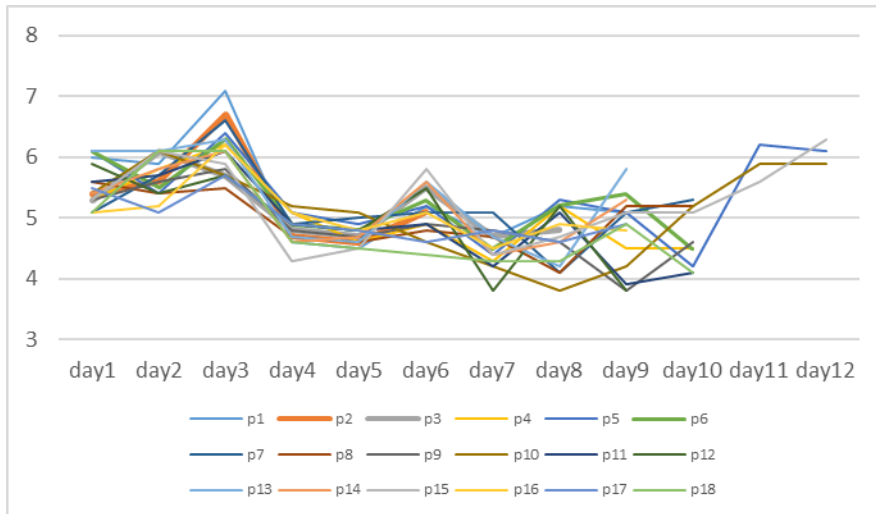


**Figure 2.** Mean salivary cortisol levels (nmol/l) among alive and deceased patients at 20 in the evening ( $p < 0.001$ )

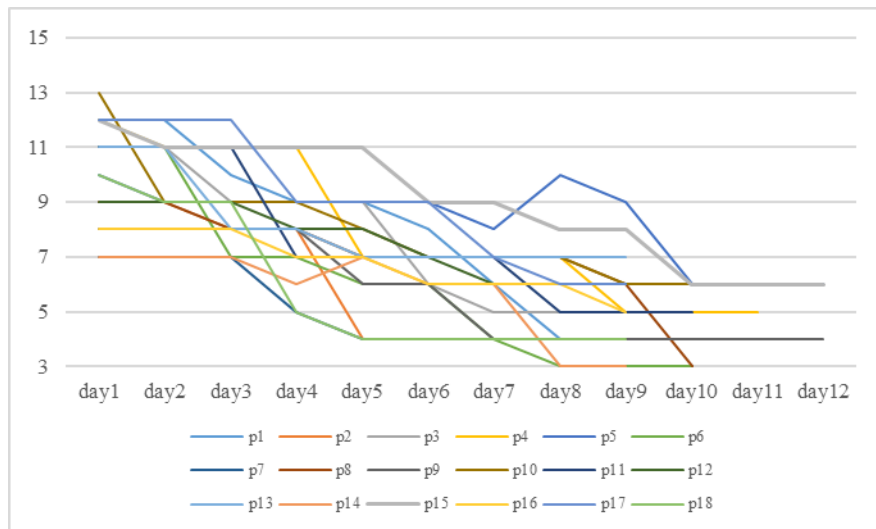
The median survival time among deceased patients was 10 days, with minimum 6 and maximum 12 days. The dynamic of salivary cortisol levels was different in the morning and in the evening for the deceased patients (Fig. 3, Fig. 4). GCS progressively decrease during the follow up period of all deceased patients (Fig. 5).



**Figure 3.** Salivary cortisol levels among deceased patients at 8 o'clock in the morning



**Figure 4.** Salivary cortisol levels among deceased patients at 20 o'clock in the evening



**Figure 5.** Glasgow coma scale among deceased patients

We found a statistically significant correlation between the increase in the morning salivary cortisol level and the decrease of the score of GCS in 11 out of 18 deceased patients (Table 2). The same correlation for the evening salivary cortisol was found in only 5 patients (Table 2).

**Table 2.** Correlation between salivary cortisol levels (in the morning and in the evening) and Glasgow coma scale)

Patients	Pearson correlation	Significance (2-tailed) p-value	Pearson correlation	Significance (2-tailed) p-value
	8 o'clock in the morning		20 o'clock in the evening	
Patient 1	-0.768	0.009*	0.470	0.171
Patient 2	-0.393	0.441	0.474	0.342
Patient 3	-0.557	0.151	0.510	0.197
Patient 4	-0.688	0.028*	0.775	0.008*
Patient 5	-0.662	0.019*	0.037	0.909
Patient 6	-0.726	0.018*	0.578	0.080
Patient 7	-0.521	0.122	0.634	0.049

Patient 8	-0.556	0.120	0.164	0.673
Patient 9	-0.755	0.012*	0.688	0.028
Patient 10	-0.663	0.037*	0.560	0.093
Patient 11	-0.767	0.010*	0.845	0.02*
Patient 12	-0.823	0.006*	0.723	0.028*
Patient 13	-0.498	0.173	0.593	0.093
Patient 14	-0.863	0.001*	0.329	0.388
Patient 15	-0.662	0.052	0.197	0.540
Patient 16	-0.788	0.012*	0.617	0.077
Patient 17	-0.515	0.156	0.769	0.015*
Patient 18	-0.423	0.000*	0.831	0.006*

\* - statistical significance using Pearson's correlation coefficient  $r$

## Discussion

### Salivary cortisol

The idea to assess salivary cortisol as a reflection of the free biologically active cortisol in the plasma is not new and was introduced in 80s (5). This method was used to study physiological stress responses and pathological syndromes (6-11). Our experience confirm the findings of different centers that salivary cortisol using ELISA is cheap, fast and reliable method to obtain information for the plasma cortisol and assess the hypothalamic-pituitary-adrenal response. We exclude less than 10% of our consecutive patients with aSAH because of problem with the numbers or timing of saliva collection. The method of saliva sampling was tested even in free-living environments and the feasibility of home-based sampling was proved (12).

### Effects of increased cortisol

Severe illnesses like aSAH usually trigger a stress response with activation of the hypothalamic-pituitary-adrenal system and the sympathetic system. There are studies linking high levels of serum cortisol to high mortality, especially in Cushing's syndrome and acute coronary syndrome (7, 11). Experimental and clinical studies found a significant acute and late increase of serum cortisol during aSAH (13, 14). Elevated levels of morning total cortisol in the acute phase of subarachnoid hemorrhage were found to be associated with a higher risk of fatal outcome and a higher rate of delayed ischemic neurological deficits (14-16). Salivary cortisol was investigated only in SAH survivors 1 year after the acute event in order to investigate the long term endocrinological sequelae.

The salivary cortisol concentrations on waking were significantly higher in SAH patients compared to the controls (17). To the best of our knowledge, we investigate for the first time the prognostic value of salivary cortisol in the acute phase of aSAH. Our results confirm the negative prognostic value of the elevated salivary cortisol both in the morning at 8.00 AM and in the evening at 8.00 PM. Moreover, we found a statistically significant correlation between the increase of the morning salivary cortisol and the decrease of the score of GCS in most of the patients who will die because of aSAH (Table 2). The need for at least morning and evening cortisol measurement was demonstrated also for serum cortisol measurements (15).

## Salivary cortisol and stress treatment

Our data confirm the presence of correlation between salivary cortisol level and bad outcome in patients with aSAH. The value of diurnal salivary cortisol measurement in neurosurgical ICU was demonstrated in acute trauma patients (18). On the other hand, sedation may diminish the level of cortisol in the serum (19). The possibilities for stress treatment and serum cortisol control in patients with aSAH may be the goal of future study. The salivary cortisol measurements seem fast, easy, and reliable method to control this treatment.

## Conclusion

The measurement of salivary cortisol in patients with aSAH provides additional information about the prognosis. Patients who will die during the acute phase of aSAH have higher morning and evening salivary cortisol compared to patients who will survive. Patients who will die have demonstrated a progressive increase of the morning salivary cortisol.

**Conflict of interest** All authors declare that they have no conflict of interest.

**Informed consent** Informed consent was obtained from all patients participated in the study.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

## References

- [1]. Oie LR, Solheim O, Majewska P, Nordseth T, Muller TB, Carlsen SM, et al. Incidence and case fatality of aneurysmal subarachnoid hemorrhage admitted to hospital between 2008 and 2014 in Norway. *Acta Neurochir (Wien)*. 2020 Sep;162(9):2251-9. PubMed PMID: 32601806. Pubmed Central PMCID: PMC7415018. Epub 2020/07/01.
- [2]. Kassell NF, Sasaki T, Colohan AR, Nazar G. Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. *Stroke*. 1985 Jul-Aug;16(4):562-72. PubMed PMID: 3895589. Epub 1985/07/01.
- [3]. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology*. 1994;19(4):313-33. PubMed PMID: 8047637. Epub 1994/01/01.
- [4]. Sherman GD, Mehta PH. Stress, cortisol, and social hierarchy. *Curr Opin Psychol*. 2020 Jun;33:227-32. PubMed PMID: 31765930. Epub 2019/11/26.
- [5]. Peters JR, Walker RF, Riad-Fahmy D, Hall R. Salivary cortisol assays for assessing pituitary-adrenal reserve. *Clin Endocrinol (Oxf)*. 1982 Dec;17(6):583-92. PubMed PMID: 6762264. Epub 1982/12/01.
- [6]. Stahl F, Dorner G. Responses of salivary cortisol levels to stress-situations. *Endokrinologie*. 1982 Oct;80(2):158-62. PubMed PMID: 6297880. Epub 1982/10/01.
- [7]. Aladio JM, Costa D, Matsudo M, Perez de la Hoz A, Gonzalez D, Brignoli A, et al. Cortisol-Mediated Stress Response and Mortality in Acute Coronary Syndrome. *Curr Probl Cardiol*. 2021 Mar;46(3):100623. PubMed PMID: 32505389. Epub 2020/06/09.
- [8]. Spiga F, Lawton MA, Lightman SL, Smith GD, Ben-Shlomo Y. Socio-demographic and psychosocial predictors of salivary cortisol from older male participants in the Speedwell prospective cohort study. *Psychoneuroendocrinology*. 2021 Oct 28;135:105577. PubMed PMID: 34823140. Epub 2021/11/26.
- [9]. Lazar L, Eisenberger NI. The benefits of giving: Effects of prosocial behavior on recovery from stress. *Psychophysiology*. 2021 Oct 22:e13954. PubMed PMID: 34676898. Epub 2021/10/23.
- [10]. Vining RF, McGinley RA, Maksvytis JJ, Ho KY. Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. *Ann Clin Biochem*. 1983 Nov;20 (Pt 6):329-35. PubMed PMID: 6316831. Epub 1983/11/01.
- [11]. Pivonello R, De Martino MC, De Leo M, Simeoli C, Colao A. Cushing's disease: the burden of illness. *Endocrine*. 2017 Apr;56(1):10-8. PubMed PMID: 27189147. Epub 2016/05/18.

- [12]. Sorensen SO, Pedersen J, Rasmussen MG, Kristensen PL, Grontved A. Feasibility of home-based sampling of salivary cortisol and cortisone in healthy adults. *BMC Res Notes*. 2021 Nov 2;14(1):406. PubMed PMID: 34727972. Pubmed Central PMCID: PMC8561883. Epub 2021/11/04.
- [13]. Nyberg C, Karlsson T, Hillered L, Stridsberg M, Ronne Engstrom E. The Early Endocrine Stress Response in Experimental Subarachnoid Hemorrhage. *PLoS One*. 2016;11(3):e0151457. PubMed PMID: 27007694. Pubmed Central PMCID: PMC4805209. Epub 2016/03/24.
- [14]. July J, As'ad S, Suhadi B, Islam AA. The association between cortisol dynamics and the course of aneurysmal subarachnoid hemorrhage. *Asian J Neurosurg*. 2011 Jul;6(2):83-7. PubMed PMID: 22347329. Pubmed Central PMCID: PMC3277075. Epub 2012/02/22.
- [15]. Poll EM, Bostrom A, Burgel U, Reinges MH, Hans FJ, Gilsbach JM, et al. Cortisol dynamics in the acute phase of aneurysmal subarachnoid hemorrhage: associations with disease severity and outcome. *J Neurotrauma*. 2010 Jan;27(1):189-95. PubMed PMID: 19772477. Epub 2009/09/24.
- [16]. Vergouwen MD, van Geloven N, de Haan RJ, Kruyt ND, Vermeulen M, Roos YB. Increased cortisol levels are associated with delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. *Neurocrit Care*. 2010 Jun;12(3):342-5. PubMed PMID: 20069390. Epub 2010/01/14.
- [17]. Poll EM, Gilsbach JM, Hans FJ, Kreitschmann-Andermahr I. Blunted serum and enhanced salivary free cortisol concentrations in the chronic phase after aneurysmal subarachnoid haemorrhage--is stress the culprit? *Stress*. 2013 Mar;16(2):153-62. PubMed PMID: 22735076. Epub 2012/06/28.
- [18]. Bartanusz V, Corneille MG, Sordo S, Gildea M, Michalek JE, Nair PV, et al. Diurnal salivary cortisol measurement in the neurosurgical-surgical intensive care unit in critically ill acute trauma patients. *J Clin Neurosci*. 2014 Dec;21(12):2150-4. PubMed PMID: 25065844. Epub 2014/07/30.
- [19]. Lindgren C, Dahlqvist P, Lindvall P, Nilsson L, Koskinen LO, Naredi S. Cortisol levels are influenced by sedation in the acute phase after subarachnoid haemorrhage. *Acta Anaesthesiol Scand*. 2013 Apr;57(4):452-60. PubMed PMID: 23167448. Epub 2012/11/22.