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Utvrđivanje vrednosti kortizola u serumu i salivi kod žena izloženih hroničnom stresu

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Determination of cortisol levels in plasma and saliva in women exposed to chronic stress

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ORGINALNI RAD (OR)
ORIGINAL ARTICLE

KRATAK SADRŽAJ

Uvod: *U literaturi je poznato da akutni i hronični stres menjaju nivo kortizola u krvi. Kao i u plazmi, nivo kortizola u salivi je pouzdan stres marker.*

Cilj: *Cilj ovog rada je bio da se odredi moguća koncentracija razlika kortizola u stimulisanoj i ne stimulisanoj salivi i plazmi kod osoba izloženih hroničnom stresu i utvrdi njihova međusobna zavisnost.*

Subjekti i metode: *U ispitivanje je uključeno 25 ispitanica ženskog pola, starosti 40 godina izloženih hroničnom stresu tokom poslednjih pet godina. Uzimani su uzorci nestimulisane i stimulisane pljuvačke i uzorci plazme u sredini radne nedelje i na polovini radnog vremena, a koncentracija kortizola je određivana ELISA – imunoeseo testom – Eclia.*

Rezultati: *Dobijeni rezultati pokazuju da se nivo kortizola ne menja u odnosu na protok salive i da se povećava tokom popodneva kod osoba izloženih hroničnom stresu. Korelacija (r) između nivoa kortizola u salivi i plazmi iznosi r = 0,91. Ne invazivna procedura omogućava da se saliva može koristiti za određivanje nivoa kortizola u situacijama kada je otežano uzimanje uzorka krvi.*

Ključne reči: Saliva, plazma, kortizol

SUMMARY

Introdiction: *It is known fact from the literature data that acute and chronic stress influence serum cortisol level. As with serum cortisol, the level of cortisol in saliva is also a reliable marker of stress.*

Material and method: *this study was performed on 25 individuals, females, age 40-50 years. The participants were exposed to long term stress, held two jobs of 8 working hours, six days a weak, domestic work was not included. All of them were high educated, and none of them were on other medication therapy*

Aim: *The Aim of this study was to evaluate possible differences in cortisol levels in stimulated and non stimulated saliva and serum and determine their association with long term stress.*

Results: *Obtained results showed that cortisol level in saliva do not depend on saliva flow rate and increases in the afternoon period in individuals exposed to chronic stress. Correlation (r) between salivary cortisol and plasma cortisol is r= 0.91. Non-invasive sampling procedure suggests that saliva may be used for cortisol measurements in situations where blood sampling is difficult to perform.*

Keywords: saliva, plasma, cortisol

Uvod

Kortizol je glukokortikoid, (sintetiše se iz holesterola), hormon koga sekretuje kora nadbubrežne žlezde a čije je lučenje pod kontrolom adenokortokotropnog (ACTH) hormona adenohipofize koga kontroliše rilizinig faktor (CRF) iz hipotalamusa.Visoke koncentracije kortizola u serumu inhibiraju lučenje CRF i ACTH mehanizmom negativne povratne sprege[1]. U krvnoj plazmi se veći deo kortizola vezuje za kortikosteroidni- vezujući globulin (CBG ili transkotin) za koji ima visok afinitet. Oko 65% kortizola se vezuje za transkotin-CBG, 30% se vezuje za albumine a 3-5% je u slobodnoj formi. Kortisol se vezuje za specifične intraćelijske receptore i utiče na brojne fiziološke funkcije, uključujući imunološke, regulaciju nivoa glukoze, tonus krvnih sudova i metabolizam kosti[2]. Lučenje kortizola je ACTH zavisan, sa najvećim nivoom ujutru a najnižim nivoom u toku noći [3]. Neuroendokrini mehanizam povećava sekreciju kortizola i do 20 puta u uslovima mentalnog stresa povećavajući ćelijski metabolizam[4-7].

Određivanjem koncentracije kortizola u ekstracelularnim tečnostima može se proveriti funkcija hipotalamo-hipofizno-adrenalne osovine[1]. Intenzitet stresa je adekvatan nivou kortizola koji se u tim situacijama izluči imajući u vidu da je stres generalizovana reakcija organizma koja ima za cilj da zaštiti organizam i spreči njegova oštećenja[7,8]. Koncentracija kortizola u salivi predstavlja 70% nevezanog kortizola u krvi koji iz krvi ulazi u salivu difuzijom kroz bazolateralnu membranu acinusa pljuvačnih žlezda. Zahvaljujući maloj molekulskoj težini i liposolubilnosti, nevezani kortizol prolazi kroz ćelijsku membranu prostom difuzijom što omogućava da se vrednost slobodnog kortizola može odrediti u svim telesnim tečnostima[3,4]. Salivarni kortizol dobro korelira sa slobodnim kortizolom iz seruma koji je biološki aktivan[3,4]. U novije vreme saliva se koristi kao dijagnostička tečnost za monitoring mnogih supstanci, pa i hormona[10]. Imajući u vidu manje invazivnu metodu uzimanja uzorka salive od uzimanja uzorka krvi, mnogi autori se slažu da bi se određivanjem kortizola u salivi izbegle nelagodnosti kod pacijenata koje nosi uzimanje uzorka krvi [6,10]. Najveća koncentracija kortizola je u jutarnji časovima a najniža uveče[11,12,13].

Iz svega navedenog nameće se pitanje: Da li koncentracija kortizola u salivi odgovara koncentraciji kortizola u plazmi?

Cilj ovog rada bio je da se odrede koncentracije kortizola u salivi (ne stimulisanoj i stimulisanoj) i plazmi kod ispitanika koji su pod hroničnim stresom i utvrdi njihova međusobna zavisnost.

Introdiction

Cortisol is a corticosteroid hormone (synthesized from cholesterol) produced by the adrenal gland whose release is controlled by the adrenocorticotropic hormone (ACTH). ACTH is controlled by the hypothalamic peptide, corticotropin releasing factor (CRF). High plasma concentrations of cortisol inhibit the release of CRF and ACTH through the negative feedback mechanism.[1] Most plasma cortisol is bound to corticosteroid binding globulin (**CBG**) due to the high affinity. About 65% of cortisol binds for transkotin-CBG, 30% for albumins and 3-5% is free. Cortisol binds to specific intracellular receptors and affects many physiological functions, including the immune system, glucose level, blood vessels and bone metabolism.[2] The releaseof cortisol is ACTH dependent with the highest levels early in the morning and the lowest levels during night.[3] Neuroendocrine mechanism increases the release of cortisol up to 20 times in mental stress conditions which also increases cellular metabolism.[4-7]

Concentration of cortisol in extra cellular fluids is a reliable marker of the hypothalamic-pituitary- adrenal feedback system.[1] Stress intensity is related to the cortisol level released in such situations because stress is a generalized body reaction aimed at protecting the organism and preventing injury.[7,8] Salivary cortisol concentration represents 70% of unbound blood cortisol which enters saliva by diffusion through the basolateral membrane of salivary gland acini. Due to its low molecular weight and liposolubility, unbound cortisol penetrates the cellular membrane by simple diffusion which enables the level of free cortisol to be determined in all body liquids.[3,4] Salivary cortisol correlates well with the free plasma cortisol which is biologically active.[3,4] Recently, saliva has been used as a diagnostic solution for monitoring hormones and other substances.[10] Given that the salivary sample collection is less invasive than blood sampling, many authors agree that determination of salivary cortisol would be more appropriate than blood cortisol.[6,10] The highest concentration of cortisol is in morning hours and the lowest in the evening.[11,12,13]

Based on the all previously mentioned, the question arises: Does the cortisol concentration in saliva correlate that in plasma?

The aim of this study was to evaluate possible differences in cortisol levels in stimulated and non stimulated saliva and plasma and determine their association with long term stress.

Subjekti i metode

Ispitanici:

U istraživanju je učestvovalo 25 zdravih ispitanika ženskog pola, starosti od 40 do 50 godina. Ove osobe su izložene hroničnom stresu poslednjih pet godina sa radnim vremenom preko osam časova, šest dana u nedelji. Ispitanice su visoko obrazovane i rade na dva radna mesta, a u obzir nisu bili uzeti kućni poslovi. Ispitanice u toku trajanja studije nisu koristile nikakve lekove.

Pre učešća u studiji, sve učesnice su bile upoznate sa procedurom i svrhom ispitivanja i potpisale su obrazac pisane saglasnosti odobren od strane Etičkog komiteta Stomatološkog fakulteta u Beogradu.

Sakupljanje uzoraka salive i krvi:

Uzorci nestimulisane i stimulisane salive kao i krvi sakupljani su u izolovanim uslovima, na sobnoj temperaturi u 14^h na polovini radnog vremena, u sredini radne nedelje (sredom). Za higijensko i efikasno sakupljanje salive korišćene su salivete (Salivete-Sarstedt,Germany^R), vaterolna iz saliveta je postavljena ispod jezika i uzorak mešovite salive sakupljan je oko 1-2 minuta (ne-stimulisana saliva) i neposredno posle žvakanja roze voska (Galenika, Srbija) u trajanju od 60 sekundi. Uzorci salive su centrifugovani na 4000 obrtaja 10 minuta i zapremina dobijena salive izražavana u ml/min. Uzorci krvi su uzimani venepunkcijom, a zatim centrifugovana na 4000 obrtaja u trajanju od 10 minuta..

Cortisol Assay

Posle dobijanja uzorka salive i krvi, koncentracija kortizola je određivana ELISA imunoesej testom-Eclia koji se zasniva na kompetitivnom vezivanju poliklonalnih antitela specifična za kortizol. Koncentracija kortizola određivana je u nmol/l. Dobijene vrednosti prezentovane su Pearsonovom korelacijom.

Rezultati

U tabeli 1 prikazane su dobijene vrednosti kortizola u plazmi, stimulisanoj i nestimulisanoj salivi. Dobijeni rezulatai pokazuju da ne postoji statistički značajna razlika između nivoa kortizola u stimulisanoj i ne stimulisanoj salivi, ali vrednosti ukupnog kortizola u plazmi su značajno različite u odnosu na vrednosti u salivi.

Subjects and methods

Participants:

This study included 25 individuals, female, age 40-50 years. The participants have been exposed to chronic stress for 5 years, held two jobs with 8 working hours, six days a week and domestic work was not included. All of them were highly educated and were not subjected to any medical treatment.

The participants in this project have given written consent after having read a detailed written description of the research approved by the Ethic Committee of the School of Dentistry in Belgrade.

Saliva and blood sample collection:

The participants were asked to collect saliva samples at room temperature in the middle of the working day (2 pm) on Wednesday (middle of the working week). Standard swabs were used (Sarstedt, Germany). In order to collect mixed saliva, the participants were asked to keep the swabs under the tongue for 1-2 minutes (non stimulated saliva) and after rose wax (Galenika, Serbia) chewing for 60 s (stimulated saliva). All samples were centrifuged at 4000 rpm for 10 minutes. Blood samples were collected by venous puncture and also centrifuged at 4000 rpm for 10 minutes.

Cortisol assay

Cortisol levels in saliva and blood samples were measured using ELISA immunoassay test – Eclia which is based on competitive binding of specific cortisol polyclonal antibodies. Cortisol concentration was expressed in nmol/l. The obtained values were analyzed using Pearson's correlation.

Results

Table 1 shows the cortisol concentrations in plasma, stimulated and non stimulated saliva. No significant differences between cortisol levels in plasma, stimulated and non stimulated saliva were observed, but the values of total cortisol in plasma were significantly different from saliva.

Tabela 1. *Koncentracija kortizola u salivi i krvnoj plazmi*
Table 1. *Cortisol concetrations in saliva and plasma*

BROJ ispitanika	Plazma (nmol/L)	NESTIMULISANA SALIVA (nmol/ml)	STIM.SALIVA (nmol/L)
1	277,0	4,27	4,34
2	406,0	5,12	5,57
3	438,0	5,54	5,69
4	278,0	4,38	4,41
5	356,0	5,25	5,19
6	420,0	5,38	5,50
7	396,00	5,59	5,51
8	328,00	4,00	3,91
9	410,0	4,15	5,00
10	325,0	4,19	4,25
11	357.0	4,95	5,46
12	375,0	5,12	4,95
13	350,0	4,15	4,60
14	425,0	5,57	5,80
15	390,0	5,48	5,75
16	325,0	4,89	5,1
17	400,0	4,12	4,25
18	360,0	4,00	4,25
19	350,0	5,00	5,05
20	375,0	5,15	5,25
21	300,0	3,85	3,90
22	397,0	3,95	4,00
23	350,0	4,10	4,25
24	250,0	3,35	3,50
25	275,0	3,15	3,67

Tabela 2 pokazuje parametrijsku Pearson- ovu korelaciju između koncentracije kortizola u serumu i salivi imajući u vidu da ove vrednosti koreliraju u odnosu na nivo ukupnog kortizola u plazmi i slobodnog kortizola u salivi. Korelacija između koncentracija kortizola u plazmi i salivi je r = 0,91. Značajnost korelacije je p< 0,05.

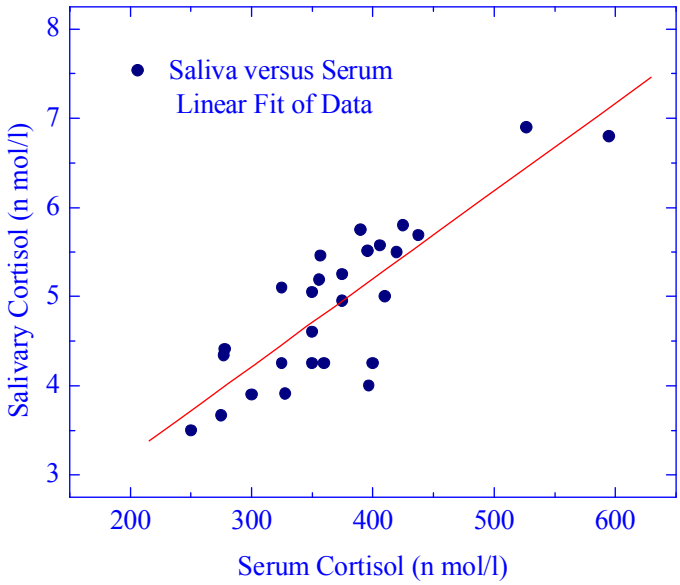
Table 2 shows the Pearson's correlation between cortisol concentrations in plasma and saliva. The Correlation factor was r=0, 91, whereas its statistical significance was p<0. 05.

Tabela 2. *Parametrijska Pearson- ova korelacija koncentracija kortizola u serumu i salivi (nmol/L)*
Table 2. *Parametric Pearson's correlation between salivary and plasma cortisol (nmol/L)*

		SERUM	Nestimulisana saliva	Stimulisana saliva
SERUM	Pearson-ova korelacija	1.000	0.708	0,910*
	Značajnost (p)	.	0.181	0.032
	N (broj uzoraka)	25	25	25
Nestimulisana SALIVA	Pearson-ova korelacija	0.708	1.000	0.387
	Značajnost(p)	0.181	.	0.519
	N (broj uzoraka)	25	25	25
Stimulisana SALIVA	Pearson-ova korelacija	0.910*	0.387	1.000
	Značajnost (p)	0.032	0.519	.
	N (broj uzoraka)	25	25	25

Grafikon 1 pokazuje linearnu zavisnost između dobijenih vrednosti nivoa kortizola u plazmi i salivi, izraženih u nmol/l.

Graph 1 shows the linear correlation of data for plasma and saliva cortisol levels given in nmol/l.



***Grafik 1**. Linearna zavisnost nivoa kortizola u serumu i salivi*
***Graph 1.** Linear correlation between cortisol levels in saliva and plasma*

Diskusija

Od ranije je poznato da je kortizol pouzdani stres marker. Tako su na primer i Fujiwara i saradnici[16] zapazili da vrednost kortizola u plazmi raste kod osoba koje su izložene hroničnom stresu na poslu u odnosu na referentne vrednosti u istom vremenskom periodu, što je prikazano i u ovom istraživanju. S druge strane, Vedhara i saradnici[15] su analizirajući koncentraciju kortizola u salivi kod studenta u ispitnom roku kada su izloženi stresu i van ispitnog roka kada je stres značajno manji, pokazali da postoji značajna redukcija u nivou salivarnog kortizola za vreme ispitnog roka. Isti autori su predpostavili da je ovo posledica povećanja kratkotrajne memorije kod ovih studenata.

Hans-Selye je tvorac adaptacionog sindroma i otkrio je ulogu ACTH i kortizola ne samo u adaptaciji na stres, već i oboljevanju[16,17]. Prema navodima Magnusa i saradnika[13], vrednosti kortizola u plazmi i salivi variraju u odnosu na doba dana (vreme uzimanja uzorka je: 8^h, 14^h i 23^h). Vrednosti kortizola u salivi kod žena i muškaraca izražena u nmol/l iznosile su: ujutru: 4,7-32,0 (8^h), posle podne (14^h): 0,00-3,60.

Discussion

It is known from the literature that cortisol is a reliable stress marker. The present results are in agreement with Fujiwara et al. who reported that plasma cortisol levels increase in individuals exposed to chronic stress at work compared to reference values. [16] On the other hand, Vedhara et al., analysing cortisol levels in students during exam periods, found the reduction in cortisol levels in saliva.[15] These authors suggested that such results could be due to students' increased short memory.

Hans-Selye is the creator of the adaptation syndrome who discovered the role of ACTH and cortisol not only in adaptation to stress but also in disorders.[16.17] According to Magnus et al., the cortisol values in plasma and saliva vary during the day (time of sample collection: 8 am, 2 pm and 11 pm). The amounts of cortisol in saliva in female and male subjects were: in the morning (8 am): 4.7-32.0 nmol/l, in the afternoon (2 pm): 0.00-3.60 nmol/l.[15]

Međutim neke japanske studije pokazuju da u nekim profesijama postoje minimalne razlike u vrednosti kortizola u odnosu na doba dana. Ovi rezultati pokazuju da su vrednosti kortizola u 14h između 3,15 i 5,80, što ukazuje na povećane vrednosti kortizola u salivi kod žena izloženih hroničnom stresu, imajući u vidu da je poluvreme eliminacije kortizola oko 100 minuta. Kirchbaum i saradnici[10] su pokazali da osobe ženskog pola imaju veću vrednost kortizola od muškaraca jer su osetljivije na stresne poslove, što nas je opredelilo da u istraživanje uključimo osobe ženskog pola.

Prema navodima Ardala i sar.[4] koncentracija salivarnog kortizola ne zavisi od protoka salive, što je potvrđeno i u našim istraživanjima.

Metod uzimanja uzorka salive je pokazan kao adekvatan i odgovarajući za određivanje nivoa kortizola u odnosu na druge metode koje se uobičajeno koriste za uzimanje uzorka mešovite salive. Iz tog razloga u ovom radu korišćene salivete, što je preporučeno kao adekvatna metoda i u radovima Poll-a i saradnika[18].

Činjenica je da su vrednosti kortizola u salivi značajno veće u odnosu na referentne vrednosti, što odgovara uzorku ispitanika koji su učestvovali u ovoj studiji. U dobijenim vrednostima nivoa kortizola u plazmi i salivi postoji korelacija, s tim što se u salivi nalazi slobodan kortizol. Teško je, za sada, reći da li metod određivanja kortizola u salivi može u potpunosti da zameni određivanje u krvnoj plazmi. Imajući u vidu da je jedino biološki aktivan slobodan kortizol, to bi se vrednosti dobijene u salivi mogle koristiti kao objektivan parametar za promene vrednosti kortizola u toku jednog dana (ujutru, u podne i uveče).

Zaključak

Može se zaključiti da ne postoji razlika u dobijenim vrednostima koncentracije kortizola u odnosu na stimulisanu i ne stimulisanu salivaciju, što znači da protok pljuvačke ne utiče na koncentraciju kortizola. Koncentracija kortizola se povećava kod ispitanika sa hroničnim stresom u toku poslepodneva. Korelacija između koncentracija kortizola u plazmi i salivi je r=0,91.

Ova neinvazivna procedura omogućava da se saliva može koristiti za određivanje nivoa kortizola u situacijama kad je otežano uzimanje uzorka krvi.

However, recent Japanese studies suggested minimal daily variations in cortisol levels in certain professions. These results showed cortisol levels between 3.15 and 5.80 at 2 pm which indicated increased cortisol values in saliva in women exposed to chronic stress, given that the cortisol elimination half-time was about 100 min. Kircbaum et al.[10] showed that women seemed to have higher cortisol levels than men probably due to the fact that women are more sensitive to stress and that was the reason to employ women only in this study.

According to Ardal et al.[4], the concentration of salivary cortisol is not dependant on salivary flow rate, which was supported by our study results.

Saliva sample collection was implemented in this study according to Poll et al. recommendation, and was found suitable for cortisol level determination in mixed saliva[18].

It is the fact that cortisol levels in saliva are much higher than reference ones and was in agreement with participants engaged in the present study. Obtained values for cortisol in plasma and saliva showed positive correlation with the exception that only free cortisol is persistent in saliva. For the time being, it is difficult to claim that the salivary cortisol determination method can substitute the plasma one. However, since salivary cortisol levels correspond only to free cortisol values which is biologically active, they may be used as an objective parameter for daily variations in cortisol levels (morning, afternoon, night).

Conclusion

The present results showed that cortisol levels in saliva was not dependant on the salivary flow rate and that there were no differences in cortisol concentrations in stimulated and non-stimulated saliva. The concentration of cortisol increased in the afternoon in individuals exposed to chronic stress. The correlation (r) between salivary cortisol and plasma cortisol was r= 0.91.

The non-invasive sampling procedure allows saliva to be used for cortisol level determination in situations where blood sampling is difficult to perform.

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