

**Study of the effect of early skin to skin contact on salivary cortisol
as a biomarker of stress in full term neonates delivered by Cesarean section**

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Abstract

Background: The transition from fetal to neonatal life is one of the most important causes of stress to the infant. When the infant attempts to cope with a stressful situation, the developing hypothalamic- pituitary- adrenal axis is activated, culminating in alterations of cortisol level. Early Skin to skin contact (SSC) between mother and her baby thought to decrease the neonatal stress. Salivary cortisol has been widely used as biomarker of hypothalamic pituitary adrenal (HPA) axis activity.

Aim of the work: Our aim is to evaluate the effect of early skin to skin contact on salivary cortisol as a biomarker of stress in full term neonates.

Methods: This case control study was conducted on 40 full term neonates with gestational age 37- 42 weeks. They were divided into two groups, the study group (n= 20) with initiation of SSC before 1st breast feeding and the control group (n= 20) with no initiation of SSC before the first breast feeding. Samples were collected from infants after delivery in both groups (The basal measurement) and 1 hour after first feed.

Results: The control group revealed a significant increase of salivary cortisol level from a mean of 24.2 ng/dl in the basal measurement to a mean of 33.4 ng/dl in the 1 hour after 1st feed measurement. In contrast, It increased slightly from a mean of 22.7 ng/dl in the basal measurement to a mean of 23.1 ng/dl 1 hour after 1st feed in the study group and this increase was not statistically significant. Cortisol level didn't statistically correlate with gestational age, maternal age or birth weight. In contrast, Heart rate and Apgar score have statistically significant correlation with it.

Conclusions: Early skin to skin contact is recommended after birth as the increase in salivary cortisol level (which is a biomarker of stress) was not statistically significant indicating less exposure to stress.

تأثير التلامس المباشر المبكر بين الأم والطفل حديث الولادة مكتمل النمو على مستوى الكورتيزول في اللعاب باعتباره مؤشر حيوي على الإجهاد

إن الانتقال من الحياة الجنينية إلى خارج الرحم هو واحد من أهم أسباب الإجهاد في الطفل حديث الولادة. فعندما يحاول الطفل التكيف مع وضع مجهد كهذا فإن المحور الوطاني- النخامي طور التطور يتحفز محدثاً تغير في مستوى الكورتيزول. غير أن التلامس المبكر المباشر بين الأم والرضيع يقلل من هذا الإجهاد وقد استخدم مستوى الكورتيزول في اللعاب كمؤشر على نشاط هذا المحور.

الهدف من هذه الدراسة البحثية هو تقييم تأثير التلامس المباشر المبكر بين الأم والطفل حديث الولادة على مستوى الكورتيزول في اللعاب كمؤشر حيوي على الإجهاد. تم اجراء هذه الدراسة على اربعين طفل حديث الولادة مكتملي النمو يزيد عمرهم الرحمي عن سبع وثلاثين اسبوع وقد قسموا الى مجموعتين كل منهما عشرين طفلاً المجموعة الأولى مجموعته الدراسه وقد تعرض فيها الاطفال للتلامس قبل الرضاعة الأولى اما المجموعة الثانية وهي مجموعة التحكم لم يتعرض فيها الاطفال للتلامس المباشر مع الام قبل الرضاعة الأولى. في المجموعتين تم قياس مستوى كورتيزول اللعاب فور الولادة وساعه بعد الرضاعة الأولى.

كانت النتيجة أن هناك ارتفاع واضح في معدل الكورتيزول في اللعاب بين القياس بعد الولادة والقياس بعد ساعة من الرضاعة الأولى وذلك في مجموعة التحكم التي لم يتعرض فيها الاطفال للتلامس. بينما كان هناك ارتفاع طفيف احصائياً في مجموعة الدراسه التي تعرض فيها الاطفال للتلامس المبكر مع الام بعد الولادة. التوصية التي خلصت اليها الدراسه هي ضرورة التلامس المباشر المبكر بين الأم والطفل حديث الولادة كوسيلة لتقليل الإجهاد له.

Introduction:

Early skin to skin contact (SSC) between mother and her baby has shown to promote positive mental and emotional health in neonates and assist in bonding and attachment between mother and her neonate. Skin-to-skin contact after birth is known to promote the infants' regulation of temperature and metabolic adaptation and maintenance of glucose blood levels compared to infants not having skin-to-skin contact or those being separated from their parents. Thus, skin-to-skin causes a down regulation of stress reactivity (Bystrova et.al. 2009).

SSC is considered a component of the term KMC, which involves kangaroo position (KP); kangaroo nutrition, based on breast feeding; and mother-family involvement (Charpak, 2015). Description of the intervention: Early SSC is the placing of the naked baby prone on the mother's bare chest at birth or soon afterwards. In the evolutionary context, this would have been "immediate and continuous". In the current care context, initiation and duration are not defined. The concept of "Care" does not change; only the place where such care is provided changes. Further, although a dose-response effect has not been documented in randomized controlled trials (RCTs), the general belief is that SSC should continue until the end of the first successful breastfeeding to show an effect and to enhance early infant self-regulation (Widstrom 2015).

On the other hand, separation of mothers from their newborn infants at birth has become standard practice, despite mounting evidence that this may have harmful effects, delivery room and postpartum hospital routines may significantly disrupt early maternal-infant interactions including breastfeeding (Anderson, 2016).

The transition from fetal to neonatal life and the separation between baby and his mother represent one of most important cause of emotional stress to the infant and may have permanent harmful effects on him (Takashi et.al., 2011). When the infant attempts to cope with a stressful situation, the developing hypothalamic-pituitary-adrenal axis is activated, culminating in alterations of cortisol level (Morelius et.al., 2015).

Salivary cortisol has been reported as useful biomarker of psychological stress and related mental or physical disease as it is noninvasive method, in addition, sample collection is standardized and easy to handle, allowing reflection of direct response to stress since biomarkers are not bound to plasma transport proteins (Voegtline and Garner, 2014).

Aim Of This Study:

Our aim is to evaluate the effect of early SSC between mother and her neonate on the salivary cortisol as biomarker of stress.

Subjects And Methods:

1. Type of the study: Case control study.
2. Study period: 6 months from March 2019 to December 2019.

This study was conducted in Alexandria police hospital, done for 40 full term neonates with gestational age higher than 37 weeks. All the

infants in this study have been subjected to detailed history taking, clinical examination and a laboratory investigation, salivary cortisol measured using ELISA.

The Subjects were divided into two groups: Group A (Control group): it is composed of 20 neonates with no initiation of early skin to skin contact before 1st feed, Group B (Study group): it is composed of 20 neonates who received early skin to skin contact before the first breast feeding.

1. Inclusion criteria: Full term neonates with gestational age 37- 42 weeks, according to guidelines of American academy of pediatrics (2004), all delivered by cesarean section only.
2. Exclusion criteria:
 1. Gestational age less than 37 weeks or higher than 42 weeks.
 2. Congenital anomalies.
 3. Baby required respiratory support.
 4. Complicated labor or normal vaginal delivery.
 5. Antepartum hemorrhage.
 6. Placental abnormalities.

Methods:

All neonates were subjected to the following:

1. Personal history: Name, age, sex, order of birth, address and consanguinity of parents. Antenatal history: maternal eclampsia, preeclampsia, maternal diabetes, antepartum hemorrhage, any placental problems or premature ruptures of membrane. postnatal history: onset of crying after birth, cyanosis or any resuscitations problems.
2. Clinical examination: General condition, Assessment of gestational age through analysis of maternal dates, Ballard score or both, Temperature, respiratory rate, weight, length, fronto-occipital circumference, complex examination including cardiac (heart rate and capillary refilling time), chest (air entry, adventitious sounds, apnea or signs of respiratory distress), Abdominal (laxity and girth for distension), neurological (suckling, Moro reflex, irritability and level of anterior fontanel) and skin (jaundice, pallor and cyanosis) examinations.
3. Salivary cortisol Samples were collected from neonates after delivery room resuscitation in both control and study groups. A second sample was collected one hour after 1st feed not preceded by Skin to skin contact in the control group and one hour after 1st feed following SSC in the study group.
4. In each time, two ml of saliva were withdrawn in 1.5 ml tube under aseptic conditions. we stored the samples at -20°C until measurement of cortisol. It was done by cortisol saliva kit enzyme immunoassay (ELISA).
5. Sample size: Sample size was calculated PASS version 11 program.

Statistical Methods:

Standard computer program SPSS for Windows, release 13.0 (SPSS Inc, USA) was used for data entry and analysis. All numeric variables

were expressed as mean [standard deviation (SD)], median, and IQR. Comparison of different variables in various groups was done using student t test and Mann Whitney test for normal and nonparametric variables respectively. Paired t or Wilcoxon signed ranks tests were used to compare multiple readings of the same variables. Chi- square (χ^2) test was used to compare frequency of qualitative variables among the different groups. Pearson and Spearman's correlation tests were used for correlating normal and non- parametric variables respectively. For all tests a probability (p) less than 0.05 was considered significant. Graphic presentation of the results was also done (Daniel, 1995).

Results:

The results of the current study were presented in tables and figures. There was no significant difference between age of control and study groups (P<0.05). Female's percentage (65% in the control group and 60% in the study group) was higher than males' percentage (35% in the control group and 40% in the study group). Figures (2).

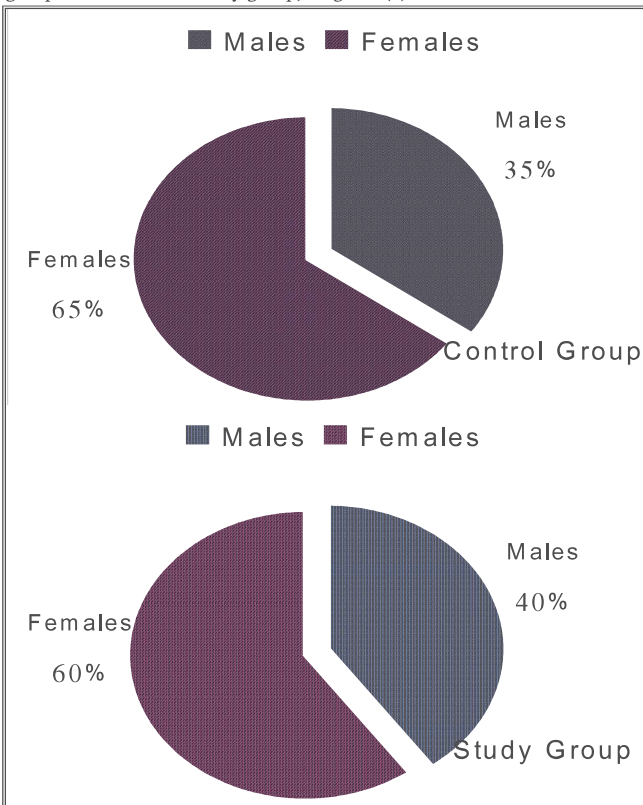


Figure (2) Gender distribution in the study groups.

There was no statistically significant difference between both groups in birth weight, maternal age or Apgar score at 1m and 5 m Table (1).

Table (1) Descriptive data of the control and study groups.

	Control group(n= 20)			Study group(n=2 0)			t- Test	p- Value	sig
	X	SD	range	X	SD	range			
Bwt In Grams	3107	295	2653- 3720	3274	327	2712- 3805	1.43	<0.05	NS
Maternal Age In Years	27	2	23- 32	26	1.5	22- 31	1.72	<0.05	NS
Apgar Score At 1 M	6.6	0.41	5- 8	6.3	0.5	6- 8	1.82	<0.05	NS
Apgar Score At 5 M	8.4	0.23	8- 9	8.00	0.41	7- 9	1.87	<0.05	NS

High percentage of mothers went to university (60% in control group and 70% in study group) and nearly half of them gave birth before (40% in the control group and 50% in the study group).

The basal cortisol level ranged in the control group from 7.8 ng/dl to 41.3 ng/dl with a mean of 24.2 ng/dl, while in the study group it ranged from 7.1 ng/dl to 39.2 ng/dl with a mean of 22.7 ng/dl. Cortisol level one hour after 1st feed ranged from 14.8 ng/dl to 54.2 ng/dl with a mean of 33.4 ng/dl in the control group, while in the study group it ranged from 7.2 ng/ dl to 39.8 ng/dl with a mean 23.1ng/dl. Table (2).

Table (2) Comparison between basal and 1h after 1st feed cortisol levels (ng/dl) in the control and study groups

	Basal cortisol (ng/ dl)			1h after feed cortisol (ng/dl)			t- Test	P value	sig
	X	SD	Range	X	SD	Range			
Control Group	24.2	7.9	7.8- 41.3	33.4	8.7	14.8- 54.2	2.6	>0.05	S
Study Group	22.7	7.4	7.1- 39.2	23.1	7.6	7.2- 39.8	0.17	<0.05	NS

No statistically significant correlation between the basal cortisol levels and gestational age, maternal age and birth weight (P value> 0.05), while correlation between the basal cortisol levels and Apgar score at 1 m, at 5 m, herat rate and respiratory rate was statistically significant (P value< 0.05). table (3).

Table (3) Correlation between basal salivary cortisol level and other variables

variable	R	P	Significance
Gestational Age	0.22	<0.05	NS
Maternal Age	0.34	<0.05	NS
Birth Weight	0.29	<0.05	NS
Apgar Score At 1m	0.51	<0.05	S
Apgar Score At 5 M	0.62	<0.05	S
Heart Rate	0.49	<0.05	S
Respiratory Rate	0.48	<0.05	S

Discussion:

The routine standard of care after a cesarean is for the baby to be taken to a warmer in the operating room, where he or she is examined, cleaned, labeled, weighted, measured, clothed, and swaddled before being shown briefly to the parents. The baby is then taken to a nursery for further assessment and observation in a warmer, while the mother is taken to a separate recovery room, with the separation typically lasting one to two hours (Császár and Bókkonac, 2018). This routine may significantly disrupt early maternal- infant interactions including breastfeeding (Anderson, 2016).

Measurement of salivary cortisol has been widely used in pediatric research for more than 20 years as biomarker of hypothalamic pituitary adrenal (HPA) axis activity during normal activity and in response to stress. Salivary cortisol is a reliable non- invasive method to assess HPA function (McCarthy et.al., 2010).

The present study was conducted on 40 full- term neonates (37- 42 weeks gestational age), they were divided into two groups, the control group with no initiation of early SSC before first breast feeding and the study group with early initiation of SSC before first breastfeeding.

We used to leave the cotton tipped applicator in the buccal mucosa of neonates for about 10- 15 minutes to get sufficient amount of saliva. There are other methods for saliva collection in neonates close to the method we used like Cignacoo et.al (2009), who found success with saliva collection in neonates by leavening the sorbettes (a type of eye spear made of

compressed cellulose sponge in mouth for 20 minutes. Another method was used to collect saliva samples in neonates by Neu et.al. (2007), who found that saliva collection device constructed with filter paper are effective for absorbing saliva from mouth. All these methods are used to collect saliva in neonates (Strazdins et.al., 2005).

Nelson et.al. (2011) Contamination of samples during collection represent issue that must be considered. One of the most common products that can contaminate saliva samples is the milk products present in infant's mouth, because both breast milk and formula contain cortisol like substance. So we found that it was better to collect samples at least 1 h after infant feeding, this agree also with.

The present study revealed no statistically significant difference between the control and study groups regarding the gestational age of infants with a mean age of 38.7 weeks (± 0.84) in the control group and 39.12 weeks (± 1.02) in the study group with P value > 0.05 . This was expected as one of the inclusion criteria in the present study was to take full term neonates and according to the guidelines of the American academy of pediatrics 2004, full term gestational age range from 37 to 42 weeks. This agree with Takashi et.al. (2011) who did study on the same gestational age with a mean of 39.9 weeks in one group of the study and a mean of 39.1 weeks in the other group.

Regarding the gender of infants in the present study, the percentage of females (65% in the control group and 60% in the study group) was higher than the percentage of males (35% in the control group and 40% in the study group).

Regarding the descriptive data in the study, there was no statistically significant difference between the both groups in the birth weight, maternal age or Apgar score at 1, 5 m. This may be attributed to the same inclusion and exclusion criteria. These demographic distribution results agree with Takashi et.al. (2011) who found also no statistically significant difference between the both groups in the birth weight, maternal age or Apgar score at 1, 5 m.

In the present study high percentage of mothers went to the university (60% in the control group and 70% in the study group) and near half of them gave birth before (40% in the control group and 50% in the study group). This helped us in our study because they were cooperative.

According to Kasser et.al. (2019), the mode of delivery could influence neonatal stress response: they found a lower cortisol reactivity among infants delivered by caesarean section compared to those vaginally born, relying on this, we considered appropriate selecting only newborns born from a caesarean section, to exclude another source of external stress.

There was statistically significant increase of salivary cortisol level from a mean of 24.2 ng/dl in the basal measurement to a mean of 33.4 ng/dl in the 1 hour after 1st feed measurement (P value > 0.05) in the control group Table (7). This in agreement with Tomerak et.al. (2017) who revealed increase in the salivary cortisol level from a mean of 35 ng/dl to 69 ng/dl. The deterioration of cortisol level in the study of Tomerak is much more when compared to our study due to the difference in measuring time as

they measured the cortisol level after birth then 4 days later, while our measurements were done after birth then 1 hour after 1st feed. The significant increase in cortisol level in the control group can be attributed to the stress effect of separation between the infant and the mother after birth. This agreed with Mohamed et.al. (2016) who reported that the stressful conditions increase salivary cortisol level. When the infant attempts to cope with a stressful situation, the developing hypothalamic-pituitary- adrenal axis is activated, culminating in alterations of cortisol level (Morelius et.al., 2015). The transition from fetal to neonatal life and the separation between baby and his mother represent one of most important cause of emotional stress to the infant and may has permanent harmful effects on him and this stress was represented by elevation in the level of salivary cortisol as biomarker of stress (Dukic et.al., 2016).

In contrast, the level of salivary cortisol increased slightly from a mean of 22.7 ng/dl in the basal measurement to a mean of 23.1 ng/dl 1 hour after 1st feed measurement in the study group (SSC group) and this increase was not statistically significant (P value > 0.05). Again this is in agreement with Tomerak et.al. (2017) who reported also a slight increase from a mean of 24.1 ng/dl in the basal measurement to a mean of 25.4 ng/dl in the second measurement after SSC sessions. The insignificant increase in cortisol level in study group (SSC) between the basal and 1h after 1st feed measurements refer to less exposure to stress and this can be explained by the effect of skin to skin contact between mother and her infant. Skin to skin contact (SSC) between mother and her baby through multi- sensory stimulation thought to promote positive mental and emotional health in neonates and assist in bonding and attachment between mother and her infant decreasing the neonatal stress after birth. It also helps in regulation of infant temperature and blood glucose level (Takahashi and Tamakoshi, 2018). This effect of SSC in decreasing salivary cortisol level in our study is similar to the result of Cignacco et.al. (2009) who found a decrease in salivary cortisol levels after kangaroo care (SSC). Our findings also confirm findings by Takashi et.al. (2011) who reported physiological and biochemical evidences that SSC is beneficial for reduction of stress during the early period after birth.

In the present study no statistically significant correlation was found between the cortisol level and gestational age, maternal age or birth weight (P value > 0.05) Table (9). This agree with Takashi et.al. (2011) results. In contrast Apgar score has statistically significant correlation with the basal cortisol level with P value < 0.05 . The higher the Apgar score the lower basal cortisol level and vice versa. The explanation of that, the lower the Apgar score the more distress of the infant and so the more release of cortisol and vice versa.

Conclusion& Recommendations:

1. We conclude the following: There was a statistically significant increase of salivary cortisol level from the basal to the 1 hour after 1st feed measurement in the control group. In contrast, the level of salivary cortisol increased slightly from the basal to the 1 hour after 1st feed measurement in the study group and this increase was not

statistically significant. The significant increase in cortisol level in the control group can be attributed to the stress effect of the separation. On the other hand, the insignificant increase in cortisol level in study group can refer to less exposure to stress and can be explained by the effect of skin to skin.

2. We recommend the following: Separation between mother and her infant after birth is not recommended as it increase the stress of neonate and may has permanent harmful effects on him. Accordingly, early skin to skin contact (SSC) is strongly recommended after birth.

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