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The impact of singing on estrogen and progesterone release among female

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Abstract

Scientific studies have suggested that singing can have positive effect on people's health. (NAFME, 2012). John Daniel Scott, has cited that people who sing are more likely to be happy because singing elevates the level of neurotransmitters which is associated with pleasure and wellbeing. Most studies on the biological effect of music has resolved around studies involving brain mapping and physiological parameters such as heart rate, blood pressure, through production of messengers such as hormones, cytokines and proteins, music elicit biological responses to stress, emotions and immune functions. Hormone certainly prove necessary for proper physiological growth and development. This study is therefore to know the influence of singing on female sex hormone release which has variety of effects on both sexual organs and diverse target tissues. This research study will be a cross sectional and controlled investigative study. Hormone will be assayed with ELIZA technique and its secretion will be determined by its concentration in the blood. The study will be carried out with a total of thirty female choral singers, grouped into three A, B, C, each group will comprise of ten subjects, using group C as control. Blood sample will collect from each group at pre and post ovulation phase of their menstrual cycle, before and after singing while the control group will not be involved in singing. The mean serum concentration in all group will be compared with group C which serve as control group.

Keywords: Estrogen; Singing; Progesterone; Hormone; Organ and Female

1. Introduction

Music may regulate the production of neurotrophins in the hypothalamus, causing reduction in stress and improved learning as well, (Angelucci et al, 2007). Not all studies concerned have produced results indicating correlations between psychological and physiological outcomes. Although patients felt less anxious after listening to music, they revealed no differences in concentrations of norepinephrine, epinephrine, cortisol, or ACTH (Migneault et al. 2004 and Wang et al, 2002). Some results have been found to conflict with each other. Music therapy increased adrenaline in one study (Hasegawa et al, 2001) but decreased adrenaline in another, (Okada et al; 2009) a result of the fact that music therapy protocols vary from one administrator to another. However, some evidence indicates otherwise as oxytocin production is increased by listening to music. Thus, psychological mechanisms influence physiological processes. More studies are needed to clarify such cause and effect relationships, (Nilsson, 2009). Most studies simply observed the direct involvement of music on messenger production by allowing participants to listen to music before measuring changes in concentrations. However, some studies combined both music and treatment with a substance in order to examine the effect of the substance which was either accentuated or reversed by music or vice versa, (Menon and Levitin; 2005and Nilsson, 2009). Active participation in producing music increased natural killer (NK) cell activity and changed gene expressions for interferon-g and IL-10, (Wachi et al, 2007). However, the participants were merely motivated by a trained mentor to play percussion instruments rather than to perform independently with musical knowledge. Musical ability has been found to influence the production of hormones (Fukui and Yamashita, 2003; Hassler, 1992 & Hassler and Gupta, 1992). Musical talent and simply listening to music have been compared with

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respect to production of IgA and cortisol, (Kreutz et al, 2004). Exploring the exact functions of cytokines, neurotransmitters, hormones, peptides, and other messengers requires further research. The aim is to determine if there is any significant difference in the serum estrogen levels among the female singing choral group in Awka Anambra state Nigeria. In its physical aspect, singing has a well-defined technique that depends on the use of the lungs, which act as an air supply or bellows; on the larynx, which acts as a reed or vibrator; on the chest, head cavities and skeleton, (Wachi et al, 2007) which have the function of an amplifier, as the tube in a wind instrument; and on the tongue, which together with the palate, teeth, and lips articulate and impose consonants and vowels on the amplified sound. Though these four mechanisms function independently, they are nevertheless coordinated in the establishment of a vocal technique and are made to interact upon one another, (Wachi et al, 2007*)*. During passive breathing, air is inhaled with the diaphragm while exhalation occurs without any effort. Exhalation may be aided by the abdominal, internal intercostal and lower pelvis/pelvic muscles. Inhalation is aided by use of external intercostal, scalene, and sternocleidomastoid muscles. The pitch is altered with the vocal cords. With the lips closed, this is called humming. The sound of each individual's singing voice is entirely unique not only because of the actual shape and size of an individual's vocal cords but also due to the size and shape of the rest of that person's body. Humans have vocal folds which can loosen, tighten, or change their thickness, and over which breath can be transferred at varying pressures(*Maton, etal 1993*).The shape of the chest and neck, the position of the tongue, and the tightness of otherwise unrelated muscles can be altered. Any one of these actions results in a change in pitch, volume (loudness), timbre, or tone of the sound produced. Sound also resonates within different parts of the body and an individual's size and bone structure can affect the sound produced by an individual.

2. Materials and methods

2.1. Sample Collection

Blood sample will be collected from the participants intravenously from their arm using sringes and needles . After collection, blood will be introduced into the heparinized tube to avoid coagulation. The blood sample collected the first participant before singing, will be labeled A1 and after singing the blood collected will be labeled A1.2,The collection of blood will continue in this sequence until the last person in this first group of ten which will be labeled A10 and A10.2 Consequently blood collected from group B will be labeled B1 and B1.2,the last person also B10 and B10.2. Both arm will be checked to locate prominent vein, a tourniquet will be tied around the upper arm to limit blood flow. The site to be used will be properly cleaned with an alcohol prepared swab.Needle will be inserted into the vein and blood collected which will be emptied into the tube.Numerous tube may be used as some type of test require different colored tubes.

2.2. Estrogen Assay Procedure

- Caution is imbibed when handling patients specimen to prevent cross contamination, the use of disposable pipette is recommended.
- All sample should be inspected for bubbles, bubbles should be removed with application stick for each sample to prevent cross contamination.
- Pipette 50ul of each calibrator, control and specimen sample into correspondingly labeled wells in duplicate.
- Pipette 100ul of the conjugate working solution into each well.
- Incubate on a plate shaker (approximately 200rpm) for one hour at room temperature.
- Wash the wells three times with 300ul of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry(the use of a washer is recommended).
- Pipette 150ul of TMB substrate into each well at timed interval.
- Incubate on a plate shaker for 10-15 minutes at room temperature (or until calibrator A attains dark blue color for desired OD)
- Pipette 50ul of stop solution into each well at the same timed interval as in step 7.
- Read the plate on a micro well plate reader at 450nm within 20 minutes after addition of the stop solution.

2.3. Progesterone Assay Procedure

Before using the calibrator leave for 5minutes on the rotary shaker. The calibrator will then be ready for use. Once opened the calibrators are stable six months at 2-8`C.

The determination of progesterone can be performed in human plasma as well as in serum. Reagent should be stored at -20^C if determination is not performed on the same day of the sample connection. Repetitive freezing and thawing of the sample should be avoided.

- The contents of each vial should be diluted with distilled water to a final volume of 500mls prior to use. Mix until crystals are completely dissolved.
- All reagent should be allowed to reach room temperature.
- Unused reagent should not be transferred into the original vials.
- It is necessary to perform the determination in duplicate in order to improve accuracy of the test result, two wells should be prepared for each point of the calibration curves, two for each control, two for each sample, one for blank.
- Add conjugate at 200ul,incubate at 37*C for one hour,then remove the content from each well ;Wash the wells 3 times with300ul of distilled wash solution.
- Add TMB substrate 100ul at room temperature22-28*C for 15 minutesin the dark.
- Add stop solution 100ul.

3. Results

Table 1 Effect of singing on the estrogen and progesterone levels of subjects

	ESTROGEN MEAN±SD	F-VALUE	P-VALUE	PROGESTERON MEAN±SD	F-VALUE	P-VALUE
Control	83.65±33.84	4.045		5.81±3.59	3.154	
Pre ovulation initial	55.92±29.99		0.02*	2.50±1.79		0.01*
Pre ovulation final	64.58±26.30		0.10	2.57±1.85		0.01*
Post ovulation initial	56.66±8.36		0.02*	4.50±2.45		0.27
Post ovulation final	39.90±17.91		0.00*	2.79±2.92		0.02*

Table 2 The Comparison of the effect of singing on estrogen and progesterone levels before and after singing for 3 hours during pre ovulation period

	INITIAL	FINAL	T-VALUE	P-VALUE
Estrogen	55.92±29.99	64.58±26.30	-2.30	0.05*
Progesterone	2.50±1.79	2.57±1.84	-0.44	0.68

Table 3 The Comparison of the estrogen and progesterone levels before and after singing for 3 hours during post ovulation period

	INITIAL Mean±SD	FINAL Mean±SD	T-VALUE	P-VALUE
Estrogen	56.66±8.36	39.90±17.91	2.60	0.03*
Progesteron	4.50±2.45	2.78±2.91	0.70	0.50

Table 4 The Comparison of the effect of the ovulation period (pre and post ovulation) on the estrogen and progesterone levels before and after singing for 3 hours during post

	PRE OVULATION	POST OVULATION	T-VALUE	P-VALUE
Extradiol initial	55.92±29.99	56.66±8.36	-0.075	0.94
Progesterol initial	2.50±1.79	4.50±2.45	-2.081	0.05*
Extradiol final	64.58±26.30	39.90±17.91	2.453	0.03*
Progesterol final	2.57±1.85	2.79±2.92	-0.943	0.35

Table 5 Correlation between serum estrogen and progesterone levels with their anthropometrics

PROGESTERONE VS	r-	P-value
Weight (kg)	0.068	0.851
Height (m)	0.035	0.923
BMI(kg/m2)	0.033	0.928
SBP(mmHg)	-0.033	0.928
DBP(mmHg)	-0.093	0.798

4. Discussion

Singing has been shown to help people ease anxiety, bring euphoria, and create a sense of belonging (Sun and Buys,2016). Studies by Sun and Buys(2016) revealed that there was a significant reduction in the proportion of adults in the singing group classified as depressed and a concomitant significant increase in resilience levels, quality of life, sense of connectedness, and social support among this group. estrogens are naturally occurring steroids secreted by ovarian follicles, the corpus luteum, the placenta, and, in small amounts, by the adrenal cortex and the testes. The secretion of estrogen is regulated by FSH released from anterior pituitary. The current study examined the effects of singing on the female ovarian sex hormones which include estrogen and progesterone among female choristers during pre and post ovulation periods respectively, it was observed that there was significant decrease in the estrogen levels of the choristers before singing at preovulation period, significant decrease in the estrogen levels of the choristers was also observed before and after singing during post ovulation period when compared to that of the control group. There was no significant difference in the estrogen levels of the choristers after singing duration at preovulation period when compared to that of the control group. The reasons for the reduction in these female hormones are yet to be ascertained. Earlier studies have shown that some hormones increase among professional singers when compared to non professional singers, it has also been observed that some other hormones decrease among professional singers, in a human study, it was found an increase in saliva of 26 healthy young men, for their secretions of oxytocin release (positive social ties hormone) and a decrease for their cortisol release (stress, anxiety, and depression hormone) after exposure to music (Wachi et al, 2007). The present study is therefore suggesting that there is a need for a further investigation to find out the reasons while there was reduction in estrogen and progesterone in the young adult females choristers when compared to young adult female non choristers.

This study observed a significant increase in the estrogen levels after singing for three hours when compared to estrogen levels before singing for three hours among young adult female choristers. This increased in estrogen levels could be attributed to the fact that singing activates the hypothalamus which will lead to release of the hypothalamic hormones. The increase in the estrogen levels as observed in this study is in agreement with some earlier studies on the effect of music on estrogen secretion. Study conducted by Fukui and Toyoshima, 2006 observed that the estrogen levels significantly increased in males after listening to chill inducing and disliked music. Fukui and Toyoshima, 2006 also reported that the estrogen levels increased with chill-inducing music but decreased with disliked music among female subjects. In an investigation on the effects of singing in a choir for 90 min in elderly female subjects (average age, 72.9 years) on hormone levels which include estrogen and testosterone. It was observed that changes in the hormone levels depended on their baseline levels, the hormone levels increased in the subjects with low baseline levels after musical activity, whereas the levels decreased in those with high baseline levels (Fukui and Toyoshima, 2006).

The observed increased in estrogen levels in this present study is not in agreement with the finding of earlier studies on the effect of music on steroid hormones. The impact of singing in a low-stress performance situation (rehearsal without an audience) and a high-stress live concert (performance in front of 610 audience members) on levels of glucocorticoids (cortisol and cortisone) was examined in 15 professional singers (Fancourt et al., 2015). The results showed a significant decrease in both cortisol and cortisone across the low stress condition, the outcome of the study suggested that singing in itself was a stress reducing (health-promoting) activity, while there were significant increases across the high-stress condition.

The result of the present study is in agreement with the observations of the previous studies on the relationship between music and oxytocin a neuropeptide. Study by Keeler et al, (2015) observed that oxytocin concentration in four singers increased after improvised singing, suggesting that better social ties lead to growth in oxytocin. Another study also observed significant increased in oxytocin concentrations in both amateur and professional singers after the

singing lesson (Grape et al., 2002). Kreutz et al (2004) observed a remarkable upward trend in oxytocin levels of a singing group when compared with a chatting group with a significant difference which indicated that singing enhances individual psychological well-being as well as induces a socio biological bonding response(Kreutz et al., 2004).

Previous studies have shown that there is a gender-specific difference in the change hormone levels when listening to music. In a study in adolescents and men in the late middle age, it was observed that the testosterone levels decreased with music they liked; whereas the levels increased with music they disliked (Fukui, 2008). In contrast, the testosterone levels were elevated in females, irrespective of the type of music (Fukui and Yamashita, 2003). However, elevated testosterone levels were also observed during dance, the study suggested that there is a relationship between music and movement (Murcia et al., 2009). The results of the study by Fukui and Toyoshima , (2003) showed that music reduced the testosterone levels in the male subjects, irrespective of the type of music. In the female subjects, Fukui and Toyoshima, (2003) also observed increased in testosterone levels with chill-inducing music whereas the levels decreased with disliked music. This present study is therefore suggesting that further studies should be carried out on the relationship between estrogen and different types of participative music (singing) on the female gender, furthermore there is a need for a study to be carried out on the effect of participative music on estrogen among male choristers of different age range, this will go a long way in finding a solution to estrogen and progesterone related medical problems that might be solved with music intervention.

5. Conclusion

In conclusion, the observations made in this study are interesting from a clinical point of view because the presented data demonstrated that estrogen and progesterone significantly increased after singing among female choristers both pre-ovulation and post-ovulation which may help clinically for females among reproductive age groups for increase in these hormones for reproduction.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was sought and obtained from the Ethical Committee of the Institution.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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