



## Serum Serotonin Levels among Homosexual and Heterosexual Men

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### ABSTRACT

**Background:** Variations in sexual preferences and orientations have both proximate and ultimate causes. Serotonin (5-HT) system is a key in the regulation of reward-related behaviors, from eating, drinking to sexual activity. Recent study demonstrated that a serotonin level is involved in sexual preference in rodent as animal models. This study focuses on the profile of serotonin levels from blood among homosexual compared to heterosexual men.

**Methods:** Eight adult ( $34.5 \pm 7.69$ ) homosexual men were purposively collected from homosexual communities in Surabaya, as well thirteen adult ( $27.61 \pm 5.14$ ) heterosexual men from Semarang. Complete psychological examinations were done, then serum serotonin levels were measured using ELISA. Furthermore age and Zung-self-rating depression scale were cross matched, then serum serotonin levels were tested using Mann-Whitney U Test to determine the difference of serotonin levels among two groups.

**Results:** Our data demonstrated that 25% of homosexual men suffered from depression. There was no difference on serum serotonin levels among homosexual men compared to heterosexual men ( $p=0.41$ ).

**Conclusion:** There is no significant difference on serum serotonin levels among homosexual and to heterosexual men.

**Keywords:** Sexual preferences, homosexual, heterosexual, serotonin, depression

### ABSTRAK

Kadar serum serotonin pada laki-laki homoseksual dan heteroseksual

**Latar belakang:** Variasi dalam preferensi dan orientasi seksual memiliki beberapa faktor penyebab. Serotonin (5-HT) merupakan kunci dalam pengaturan perilaku mulai dari makan, minum sampai aktivitas seksual. Penelitian terdahulu menyebutkan bahwa kadar serotonin memiliki peran dalam penentuan preferensi seksual pada tikus. Penelitian ini bertujuan untuk menganalisis kadar serotonin darah pada laki-laki homoseksual dan heteroseksual.

**Metode:** Delapan laki-laki homoseksual dewasa secara purposif ( $34,5 \pm 7,69$  tahun) diambil dari komunitas homoseksual di Surabaya dan 13 laki-laki heteroseksual dari Semarang ( $27,61 \pm 5,14$  tahun), kemudian dilakukan skrining tanda depresi dengan menggunakan Zung-self-rating depression scale. Kadar serotonin serum diperiksa dengan menggunakan metode ELISA. Data yang diperoleh kemudian dianalisis dengan menggunakan uji Mann-Whitney U untuk melihat perbedaan kadar serotonin serum pada kedua kelompok.

**Hasil:** 25% laki-laki homoseksual mengalami depresi, dan tidak ada perbedaan kadar serotonin pada serum laki-laki homoseksual maupun heteroseksual ( $p=0.41$ ).

**Simpulan:** Tidak terdapat perbedaan antara kadar serotonin serum laki-laki homoseksual dengan heteroseksual.

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## INTRODUCTION

Anomalous sexual preferences which is refer to how an individual would like to reach orgasm<sup>1</sup> and sexual orientations which is defined as the patterns of sexual thoughts, fantasies, and attractions that an individu has toward other persons of the same or opposite gender result in complicated medical and non medical aspect problems which are should be solved. Medical aspects such as odds of sexual transmitted diseases-syphilis, genital wart, human immunodeficiency virus (HIV) infection and human papilloma virus (HPV) infection-need special attention for its treatments.<sup>2-4</sup> In addition, both sexual preferences and orientation also bring psychological matters because lesbian, gay and bisexual (LGB) people are subject to institutionalized prejudice, social stress, social exclusion (even within families) and anti-homosexual hatred and violence and often internalize a sense of shame about their sexuality.<sup>5-7</sup> LGB people appear to be at greater risk than heterosexual people for mental disorders and suicidal behavior<sup>5</sup> whereas a lesbian, gay, or bisexual identity was associated with higher odds of any mood or anxiety disorder for both men and women, women reporting only same-sex sexual partners in their lifetime had the lowest rates of most disorders.<sup>5,8</sup>

Variations in sexual preferences and orientations have both proximate and ultimate causes. Proximate causes include genetically initiated events, brain structures, and learning (experience-induced neural changes). Ultimate causes refer to the features of ancestral environments that were the agents of selection producing the species-typical reproductive behavior we observe today.<sup>1</sup>

The hypothesis that hormones exert similar influences on human neurobehavioral development has been debated, but recent studies provide convincing evidence that prenatal androgen exposure influences children's sex-typed play behavior.<sup>9</sup> The neurohormonal events involve in producing male homosexual erotic preferences also result in behavioral, cognitive and neuroanatomical features that are often, but not always, more typical of women than men.<sup>1</sup> Research in mammals has demonstrated that pheromone sensing in the periphery is important for sexual preference.<sup>10</sup>

The brain's serotonin (5-HT) system is a key in the regulation of reward-related behaviors, from eating and drinking to sexual activity.<sup>11</sup> Previous studies in mammals have implicated 5-HT in male sexual behaviors such as erection, ejaculation and orgasm in mice and humans. Depletion of 5-HT by treating animals with p-chlorophenylalanine (pCPA) or tryptophan-free diets induced male-male mounting.<sup>11</sup> Recent study demonstrated that the central serotonergic

signaling was crucial for male sexual preference in mice.<sup>10</sup>

Despite of a large number studies regarding role of serotonin on sexual behavior, only few study has been demonstrated to have the role in sexual preference, it was only one published study demonstrated the role of serotonin levels on sexual preference using animal models, and there is no study regarding the role serotonin levels on sexual preference and orientation using homosexual men as subject of study.

This study focuses on the profile of serotonin levels from blood among homosexual and heterosexual men related with severity of depression which may be important to enhance the clinical management effort of clinician and influence the concept of researchers who are investigating neurobiology of sexual preferences and orientation.

## METHODS

A cross sectional study in the field of psychiatry, correlated with neurology and biochemistry was done in Semarang and Surabaya, Indonesia for a year. All of data collections and study will be carried out with permission of the ethical committee Faculty of Medicine Diponegoro University/Rumah Sakit Dr. Kariadi Semarang Indonesia. Adult homosexual men were purposively collected from homosexual communities in Surabaya, Indonesia as well as heterosexual men from Semarang. The subjects whom diagnosed with homosexual-men who have patterns of sexual thoughts, fantasies, and attractions that an individual has toward other persons of the same gender- and heterosexual-men who have patterns of sexual thoughts, fantasies, and attractions that an individual has toward other persons of the opposite gender-based on their responses on questionnaires for sexual preferences and orientations, were invited by letters to join to this study. Complete psychological examinations were done. The data including personal identity of each subject-age, occupation, ethnic, marital status, education and demographic localization-, data on clinical signs of depression, and serum serotonin levels were collected.

Classification of signs of depression were obtained using Zung Self-rating Depression Scale, then classified into two groups-undepressed, and depressed based on the scores. In order to measure blood serotonin levels, venous blood were collected directly after all of psychological assessment, and serum serotonin levels were measured using ELISA.

Blood preparation, 5 ml venous blood were collected, and then incubated for 30-45 minutes until coagulated. Afterward, blood were centrifuged on 3000 rotation per

minute (rpm) for 15 minutes. Supernatants (serum) were collected then stored at minus 20°C. 25 µl serum were added with acylation buffer and acylation reagent then mixed for 15 minutes at room temperature (20-25°C).

Measurement of serum serotonin levels, serum serotonin levels were measured using Serotonin ELISA fast track from Beckman Coulter (ref. BA E-8900). Twenty five microliter acetylated standards, controls and samples were added with 100 µl serotonin antiserum into the appropriate wells then incubated for 30 minutes at room temperature on a shaker (approximately 600 rpm). The contents of wells were discarding 3 times using 300 µl wash buffer, and dried. A hundred microliter enzyme conjugate was added into wells and incubated for 15 minutes at room temperature on a shaker. The contents of wells were discard 3 times using 300 µl wash buffer, dried and added with 100 µl substrate then incubated for 15 minutes at room temperature on shaker. One hundred microliter stop solutions were added to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution. The absorbances of solutions were read within 10 minutes using a microplate reader on 450 nm and a reference wavelength between 620 nm and 650 nm. Quantification of samples serotonin levels were achieved by comparing their absorbance with a reference curve prepared with known standard concentrations. Data analysis, furthermore the data were analyzed using SPSS 17 for Windows. First of all, age and sign of depression that obtained by Zung Self-

Rating Depression (ZSRDS) cross matched among homosexual and heterosexual groups. Data of serum serotonin levels were tested to determine the difference of serum serotonin levels at  $\alpha=0,05$ .

**RESULTS**

We examined eight adult (34,5±7,69) homosexual men that were purposively collected from homosexual communities in Surabaya, as well thirteen adult (29.8±4.47) heterosexual men from Semarang. Ages of respondents were tested to see whether there were differences between homosexual and heterosexual groups. The result showed that there were no significant difference on age of respondents (p=0.064). Table 1 shows our result for age and serum serotonin levels.

Sign of depression among respondent were tested using the Zung Self-Rating Depression Scale. Our result demonstrated that in homosexual group 25% of respondent suffered from depression as well 7.7% of heterosexual group. However, using X<sup>2</sup> test, our result suggested that there was no significant difference for sign of depression among these two groups (p=0.63).

Finally, the serum serotonin levels were tested using ELISA Kit for Serotonin, and it was demonstrated that even there was a slight difference on mean of serum serotonin levels among homosexual (132,12±56,91) compared to heterosexual group (151,53±4.47), but by using Mann-Whitney U test, it showed that there was no significant difference on serum serotonin levels (p=0.41).

Table 1. Age and serum serotonin levels among homosexual and heterosexual men

Parameter		Means ± SD	p value
Age (years)	Homosexual	34.5 ± 7.69	0.06 *)
	Heterosexual	29.8 ± 4.47	
Serum serotonin levels (ng/ml)	Homosexual	132.12 ± 56.91	0.41**)
	Heterosexual	151.53 ± 4.47	

\*) Independent T Test

\*\*) Mann Whitley U Test

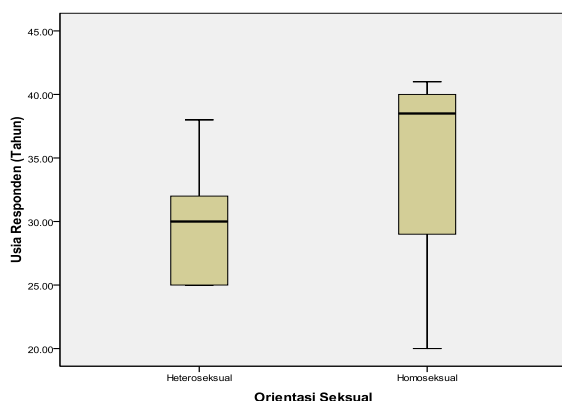


Figure 1. Boxplot of age from respondents

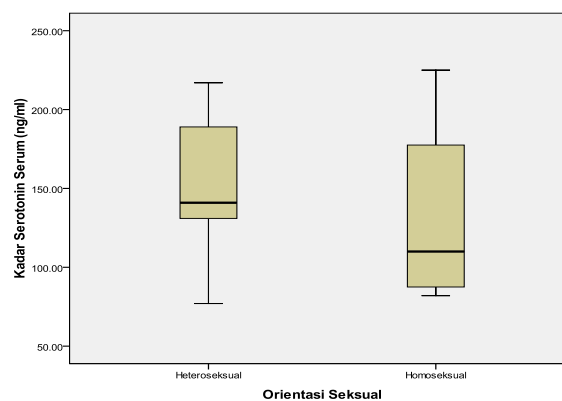


Figure 2. Boxplot of serum serotonin levels among homosexual and heterosexual men

## DISCUSSION

Depression which is described in detail by Jackson is a common mood disorders which carries it a high degree of morbidity and mortality.<sup>12</sup> It is a serious disorder that interferes more with physical and mental functioning than other chronic condition such as hypertension, arthritis and diabetes.<sup>13</sup> Depression is highly prevalent among people who commit suicide.<sup>14</sup> Major depression significantly affects the quality of life and productivity of the patient. Early recognition and treatment are essential in order to minimize the personal and societal cost associated with depression. Treating depression requires an understanding of the gravity of the illness and thorough consideration of the individual factors of each case.<sup>13</sup>

As mentioned before that anomalous sexual preferences and orientation bring psychological matters. Lesbian, gay, or bisexual (LGB) identity was associated with higher odds of any mood or anxiety disorder for both men and women.<sup>6,8</sup> Our result suggested that 25% of homosexual, suffered from depression. There were several reasons for suffering of this disorders such as lesbian, gay and bisexual (LGB) people were subjects to institutionalized prejudice, social stress, social exclusion (even within families) and anti-homosexual hatred and violence and often internalize a sense of shame about their sexuality.<sup>5-7</sup>

Variations in sexual preferences and orientations have both proximate and ultimate causes. Proximate causes include genetically initiated events, brain structures, and learning (experience-induced neural changes). Ultimate causes refer to the features of ancestral environments that were the agents of selection producing the species-typical reproductive behavior we observe today.<sup>1</sup>

The hypothesis that hormones exert similar influences on human neurobehavioral development has been debated, but recent studies provide convincing evidence that prenatal androgen exposure influences children's sex-typed play behavior.<sup>9</sup> The neuro-hormonal events involved in producing male homosexual erotic preferences also result in behavioral, cognitive and neuroanatomical features that are often, but not always, more typical of women than men.<sup>1</sup> Research in mammals has demonstrated that pheromone sensing in the periphery is important for sexual preference.<sup>10</sup>

The direction of erotic gender preference in men appears to be determined by neurohormonal intra-uterine events, although the details of this process remain to be established. Both genetic and developmental (the fraternal birth order effect) antecedents to these neurohormonal changes have been found, but the relationship between these classes of antecedents remain obscure.<sup>1</sup>

The brain's 5-HT (serotonin) system is a key in the regulation of reward-related behaviors, from eating and drinking to sexual activity.<sup>11</sup> The neurotransmitter 5-HT has been implicated in male sexual behaviors such as erection, ejaculation and orgasm in mice and humans. Previous studies in mammals have implicated 5-HT and dopamine in male sexual behaviors.<sup>10</sup> Depletion of 5-HT by treating animals with p-chlorophenylalanine (pCPA) or tryptophan-free diets induced male-male mounting. However, pCPA treatment was thought to increase sexual activity where as its effect on sexual preference has not been investigated. Interpretation of pCPA results was complicated further by the lack of specificity: pCPA may affect noradrenalin and dopamine at higher concentrations.<sup>10</sup>

Recent study showed that the central serotonergic signaling is crucial for male sexual preference in mice.<sup>10</sup> Liu *et al* (2011) using genetically modified mouse result in lack of tryptophan hydroxylase 2 (*Tph2*) demonstrated that the central serotonergic signaling is crucial for male sexual preference in mice. This is the first time, to our knowledge, that a neurotransmitter in the brain has been demonstrated to be important in mammalian sexual preference.<sup>10</sup>

In contrast to previous study, by analyzing level of serum serotonin, our results suggested that there was no significant difference of its levels among homosexual men compared to heterosexual men. However, these results did not mean that serotonin was not involved in sexual preferences and orientation. As we know, some serotonin is produced by the decarboxylation and hydroxylation of L-tryptophan, and nowadays it is known that there are two TPH enzymes, TPH1 and TPH2, which define two independent 5-HT systems. TPH1 generates more than 95% of the bodily 5-HT in the gut, from where it is transported by platelets to all organs except the brain since it cannot cross the blood-brain barrier. In the brain, only TPH2 is exclusively responsible for the first step of 5-HT synthesis.<sup>15</sup> It is hypothesized that maybe only serotonin which synthesized in the brain that has role for sexual preference and orientation.

## CONCLUSION

There is no significant difference on serum serotonin levels among homosexual compared to heterosexual men.

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