**OXTR Gene mRNA Expression is Correlated to Prosocial Behavior of Children in the Golden Generation Program of Nusa Tenggara Barat**

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**Abstract**

**BACKGROUND:** Cases of undernutrition, such as stunting and wasting, in Nusa Tenggara Barat (NTB), Indonesia, was found to be relatively high. Therefore, local government launched a golden generation program called GEN NTB to improve the quality of human resources by achieving a healthy, intelligent, devout, and productive generation in 2045. One of the genes known to be related with prosocial behavior is the oxytocin transferase (**OXTR**) gene. This study was conducted to determine the association between **OXTR** gene mRNA expression and prosocial behavior of the GEN NTB children.

**METHODS:** This was an analytical observational case-control study involving 25 children as GEN NTB samples and 26 children as controls. Blood samples were tested for OXTR protein level with enzyme-linked immunosorbent assay (ELISA), and **OXTR** mRNA expression with real time polymerase chain reaction (RT-PCR). Prosocial behavior was characterized and determined by using a rating method, which valued from 1 to 4 for poor to very good behavior.

**RESULTS:** The average OXTR protein levels of the GEN NTB group was 88.28 ng/mL, which were higher than the average OXTR protein levels of control group (2.41 ng/mL). According to fold change analysis, the **OXTR** mRNA expression in GEN NTB group was also higher than the control group (10.91 vs. 6.40). Interestingly, observations on the prosocial behavior of the GEN NTB group showed significantly higher rate values compared to the control group (17.3 vs. 8.0, \(p=0.034\)). Hence, these findings showed that the OXTR protein level and **OXTR** mRNA expression was correlated with the better prosocial behavior.

**CONCLUSION:** Higher rating of prosocial behavior of the GEN NTB children is related to the higher **OXTR** mRNA expression levels. This might be attributed to the interventions of GEN NTB program that may elevate children's quality of life since early childhood.

**KEYWORDS:** GEN NTB, OXTR protein, mRNA expression, prosocial behavior, children

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their surrounding environment. To ensure that the future generation have better quality of life, it is important to recognizing the prosocial behaviour in children since early age.

In 2016, the local government launched a social innovation program called Golden Generation of Nusa Tenggara Barat (GEN NTB) to elevate the quality of human resources in 2045. The aims of this program were to systematically develop a religious, healthy, intelligent, and productive generation in NTB Province.(2) Newborns whose mothers are included in this program will be followed prospectively until their 1000 days after birth. The GEN NTB program consequently provides adolescent health services, comprehensive and quality pregnancy check-ups, supplementation of micronutrients for pregnant women, regular examination of child growth and development, infant and child feeding program, as well as environmental health maintenance.(2,5) These conditions and stimuli are needed during children early development period to achieve their optimum development.(6)

The oxytocin receptor (OXTR) gene is previously reported to have a strong correlation to social behavior in children. The OXTR gene showed a contribution to empathy which refers to the ability to understand and share other people’s internal states or responses.(7,8) However, there have been very limited publication on the effect of OXTR in the stunting area where the specific program has been conducted. Hence, this study was conducted to explore the association between prosocial behavior and OXTR gene mRNA expression in children aged 4-6 years in the GEN NTB program.

### Methods

#### Study Design
This was an observational case-control study with non-probabilistic purposive sampling. The ethical clearance was granted by The Research Ethics Committee of the Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia (Approval No: 409/UN4.6.4.5.31/PP36/2021, Protocol No.: UH21050322). The study was designed to analyze the association between OXTR mRNA, OXTR protein level and prosocial behavior of children aged 4-6 years by using reverse logic.

#### Subjects Recruitment
The study took place in three locations in NTB Province, namely Lombok Tengah and Lombok Timur, where GEN NTB program have been applied in since 2016; as well as Mataram, a city with similar socio-economic status as the previous two locations. The subjects were recruited from kindergartens in each locations during November to December 2021.

The cases group subjects were recruited from Lombok Tengah and Lombok Timur. Inclusion criteria for the cases group were children aged 4-6 years old whose mother was the target for NTB GEN program, and the exclusion criteria were children who dropped out of the program, no longer lived in the study area, or did not agree to be involved in the study. On the other hand, the control group subjects were children aged 4-6 years old living in Mataram. Finally, as much as 25 children were recruited as the case subjects, and 26 children were recruited as the control subjects.

#### Prosocial Characterization
The nutritional status of subjects was determined based on the national anthropometry definition for stunting and body mass index (BMI). The assessment was carried out by comparing the measurements results of weight and height with the Child Anthropometric Standards according to the World Health Organization (WHO) Child Growth Standards for children aged 0-5 years.(9) The determination was conducted by measuring the Z-score = measured value – a median of reference population/standard deviation of the reference population. Stunting was defined as children whose height was less than −2 SD; and wasting was defined as children whose BMI was less than −2 SD.

\[
Z_{\text{score}} (\text{Stunting}) = \begin{cases} 
\frac{H - H_{\text{med}}}{H_{\text{med}} - H_{(\text{table}-1SD)}} & \text{for } H < \text{median} \\
\frac{H_{(\text{table}+1SD)} - H_{\text{med}}}{H_{\text{med}}} & \text{for } H > \text{median} 
\end{cases}
\]

\[
Z_{\text{score}} (\text{BMI}) = \begin{cases} 
\frac{\text{BMI} - \text{BMI}_{\text{med}}}{\text{BMI}_{\text{med}} - \text{BMI}_{(\text{table}-1SD)}} & \text{for } \text{BMI} < \text{median} \\
\frac{\text{BMI}_{(\text{table}+1SD)} - \text{BMI}_{\text{med}}}{\text{BMI}_{\text{med}}} & \text{for } \text{BMI} > \text{median} 
\end{cases}
\]

Note: \( H \) = height, \( \text{BMI} = \frac{\text{weight}}{\text{height}^2} \)

Subjects' prosocial characterization was defined by the observed behavior criteria of helpfulness, friendship, sharing, cooperation, and caring.(10,11) These criteria were rated from 1 to 4 for poor to very good behavior. Therefore, the total rating of five prosocial behavior criteria would be obtained for each group of subjects. This observation was performed by the standardized officer from Provincial Health Office of NTB.
Blood Samples Collection
As much as 0.3 mL of venous blood samples were taken from subjects to determine OXTR protein and OXTR gene mRNA expression levels. The blood was put into a tube containing 900 uL of L6 solution consisting of 120 g guanidium thiocyanate (GuSCN), then was stored in a freezer at the Microbiology Laboratory, Universitas Mataram. This storage was set up at a room temperature of 20°C for seven days prior to the examination at the Molecular Biology and Immunology Laboratory, Faculty of Medicine, Universitas Hasanuddin. The serum was separated by centrifuge at 3000 rpm for 15 minutes, and then the material will be used for examination.

Nucleic acid was isolated by using GuSCN method, then nucleic acid quality was tested using a fluorocytometer. Furthermore, the nucleic acid would be combined with the silica or diatom particles contained in the reagent.(12)

OXTR mRNA Quantification
OXTR mRNA quantification was performed using real time polymerase chain reaction (RT-PCR) methods with CFX Connect System (Bio-Rad, Hercules, CA USA) based on literature.(13) The PCR conditions were following the previous research protocol (14,15), with forward primer sequence: GCAGGGGCATCCCAACTCG; and reverse sequence: AAAATGAGCGGGAATCCTCTACC. For internal control, a housekeeping gene GAPDH was used, with the forward primer sequence: GAAATCGCCAATGCCAACTC; and reverse sequence: AAAATGAGCGGGAATCCTCTACC. The primers for OXTR were synthesized by Macrogen Inc (Seoul, South Korea).

PCR conditions were started with an initial reverse transcriptase temperature of 48°C for 30 minutes and followed by PCR activation at a temperature of 95°C for 10 minutes, then followed by temperatures of 95°C for 15 seconds and 58°C for 60 seconds as much as 45 cycles,(16,17) The amplification process used specific oligonucleotide primers, where the housekeeping gene used was GAPDH.(18,19) The qRT-PCR used the SYBR Green qRT-PCR Master Mix Kit, single stage (Applied Biosystems, Waltham, MA, USA). The protocol was adjusted using the instrument by changing the dye dilution according to the manual instructions and following the manufacturer's recommended instrument for the RT-PCR cycle program.

OXTR Protein Level Examination
Examination of OXTR protein level used enzyme linked immunosorbent assay (ELISA) method, following the kit instruction (Catalog No: LS-F6333, LifeSpan Bioscience Inc, Seattle, WA, USA). The examination was conducted in duplicate to ensure the validity of the ELISA results. The first step was the addition of 100 uL of assay diluent containing protein buffer into each well, then, 100 uL of standard fluid containing the target recombinant human protein OXTR from the pre-determined kit was added. Incubation of the diluted liquid was conducted for 2 hours at room temperature, followed by the washing process with sterile phosphate buffered saline (PBS) for 4 times in a row. Subsequently, the addition of 200 uL conjugate liquid containing streptavidin horseradish peroxidase (HRP) into each well was conducted, and liquid samples were covered and incubated for 2 hours at room temperature. The liquid was sucked and then rewashed for 4 times using sterile PBS. Finally, the last process was the addition of 200 uL substrate solution containing 3,3’,5,5’-tetramethylbenzidine (TMB) into each well and read using ELISA Reader 270 (Biomerieux, Marcy-l’Etoile, France).

Statistical Analysis
The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) (IBM Corporation, Armonk, NY, USA). Kolmogorov-Smirnov test was performed for data normality test, with p>0.05 showed that the data was normally distributed.

To determine whether there was an association between independent variable and dependent variable in this study, bivariate analysis was carried out using the Pearson Product Moment Test, with a significance level of p<0.05. The multivariate analysis was used to determine the relationship of significance level between several independent variables and the dependent variable with the ANOVA test (p<0.05 was considered as significant).

Results

Characteristics of the Subject
Among GEN NTB group, there were 15 males (60%), while in the control group there were 13 males (50%). Statistical analysis did not show a significant difference (p=0.477) for the gender criteria. Physical characteristics showed that stunting subjects were found among subjects in the GEN NTB group (7 out of 25, 28%) and control group (2 out of 26, 7%) (Table 1).

According to the national anthropometry standard, the mean stunting index (Z-score) of the GEN NTB group was -1.73±1.28 SD. On the other hand, the mean for the control group was -0.56±0.87. The lowest index was found in the
Table 1. Characteristics of subjects in terms of stunting and wasting conditions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>GEN NTB Group (n=25)</th>
<th>Control Group (n=26)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td>0.477</td>
</tr>
<tr>
<td>Male</td>
<td>15 (60%)</td>
<td>13 (50%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>10 (40%)</td>
<td>13 (50%)</td>
<td></td>
</tr>
<tr>
<td>Stunting (Z-score), Mean±SD</td>
<td>-1.73±1.28</td>
<td>-0.56±0.87</td>
<td>0.046*</td>
</tr>
<tr>
<td>BMI (Z-score), Mean±SD</td>
<td>0.17±1.78</td>
<td>-0.82±1.62</td>
<td>0.025*</td>
</tr>
</tbody>
</table>

* p-value < 0.05 is considered as significant, tested with Mann-Whitney.

GEN NTB group, which belong to the very short category (severely stunted) (Z-score < -3.00 SD). Meanwhile the lowest index of the control group was classified in a short category (Z-score < -2.00). The statistical analysis with the non-parametric test using Mann-Whitney found a significant difference in the mean stunting score among the GEN NTB group and the control group (p=0.046) (Table 1).

Based on the category of less wasted children, there were 4 wasted subjects of a total of 51 (7.8%) subjects observed. According to the BMI Z-score, the mean index for the GEN NTB group was 0.17±1.78 SD, while for the control group was -0.82±1.62. The lowest Z-score index in the GEN NTB group was -2.93 (wasted), which was close to the Z-score of -3.0 SD (severely wasted). On the other hand, the lowest index in the control group was -2.52 (wasted). A significant difference was found in the wasting conditions between the GEN NTB group and the control group (p=0.025) (Table 1).

Prosocial Behavior
Prosocial behavior was rated in four categories: 1 (poor), 2 (fair), 3 (good), and 4 (very good), for following five consecutive criteria: helping, friendship, sharing, cooperation, and caring. Each criterion was examined according to a specific lesson in each grade in the kindergartens of both groups. The prosocial total rating was then calculated for subjects in both groups. The average total rating for the GEN NTB group was 17.8, while the control group was only 8.0. The lowest total rating for the GEN NTB group was 11, and for the control group was 5. While, the highest total rating for the GEN NTB group was 20, and 13 for the control group. The median value of the average total rating for the GEN NTB group was higher, with the rating of 3.8, when compared to the control group, with the rating 1.6. These values, therefore, indicated that the prosocial behavior of the GEN NTB group was categorized as very good behavior; whereas, the children in the control group was categorized to have a poor-fair behavior.

OXTR Gene Protein and OXTR mRNA Expression
Examinations of OXTR gene protein levels and OXTR mRNA expression were conducted in both groups. It was found that OXTR protein and OXTR mRNA expression in the GEN NTB group were higher than the control group. The difference in OXTR protein levels between the GEN NTB group and the control group was 61.87 ng/mL. The mean OXTR protein levels of the GEN NTB group and control group were consecutively 88.28±28.70 ng/mL and 26.41±18.26 ng/mL. Statistically, the non-parametric test with Mann Whitney showed a significant difference of OXTR gene protein levels between GEN NTB group and the control group (p<0.001) (Table 2, Figure 1).

Correspondingly, the OXTR mRNA expression in the GEN NTB group were higher than the control group. The difference between the two groups was 4.51 according to the fold change analysis. The mean mRNA expression of the GEN NTB group were 10.91±1.87, whereas in the control group were 6.40±1.33. There was a significant difference of OXTR mRNA expression between the two groups (p<0.001) (Table 2, Figure 2).

Table 2. Test results of OXTR protein, OXTR mRNA expression, and prosocial behavior.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEN NTB group (n=25)</td>
<td>Control Group (n=26)</td>
<td></td>
</tr>
<tr>
<td>OXTR Protein (ng/mL)</td>
<td>88.28±28.70</td>
<td>26.41±18.26</td>
</tr>
<tr>
<td>OXTR mRNA (fold change)</td>
<td>10.91±1.87</td>
<td>6.40±1.33</td>
</tr>
<tr>
<td>Prosocial Behavior (score)</td>
<td>17.80±2.90</td>
<td>8.00±1.70</td>
</tr>
</tbody>
</table>

*p-value < 0.05 is considered as significant, tested with Mann-Whitney.
OXTR Gene mRNA and Prosocial Behavior of GEN NTB Children (Isnaeni W. et al.)
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Discussion

The results of the present study showed that prosocial behavior of the GEN NTB children was very good (82.1%). This could be due to the intervention of the program since their early childhood life. The intervention included sufficient nutrition provided by the government for the GEN NTB children. Eventually, stunting was still found by physical observation, however higher number of normal children were found in both groups. These findings may be related to several factors, such as their mothers were too young to give birth (under 20 years), had birth period too close one to another (less than 2 years), were too old to give birth (over 35 years), or had too many children (more than 4 children).(1,2) Due to these problems, some children might have inadequate nutrition intake.(20)

It is well known that good fetal growth and development were supported by several factors, not only adequate nutrition, but also hormonal factors and the environment around the fetus.(21) Various treatments provided in the GEN NTB program, that are given to the mother and their newborn until the babies reached 1000 day, are important for personal and social development for the children, and not only intended for the physical and fitness development of the children.(22-26)

A breastfeeding process will assist the formation of neurotransmitters in the form of tryptophan as the raw material for making neurotransmitters such as catecholamines and serotonin for the babies. Both neurotransmitters contribute to the focus of attention and maintain emotions and prosocial behavior in children.(27) In this process, several components of essential nutrients, such as carbohydrates, amino acids, and important vitamins, can affect the changes in gene activity and health.(27) Thus, tryptophan can increase the mRNA expression of the OXTR gene and the OXTR protein level produced through
Figure 4. Scattered plot on the relationship of OXTR mRNA expression and prosocial behavior.

the posterior pituitary in the form of oxytocin (OXT) that binds to OXTR.(28)

Although the case of stunting was higher in the GEN NTB group compared to the control, the BMI Z-score was better in the GEN NTB group than in the control. These better nutritional treatments might, therefore, result in better health physical conditions, since relatively high OXTR protein levels were found in the GEN NTB children. The OXTR protein levels of the GEN NTB group were greater than that of the control group. The difference between the two groups was 61.9 ng/mL; similarly, the difference in OXTR mRNA fold change expression level was 4.5 ng/mL between the GEN NTB group and control group. These results indicated that OXTR mRNA expression is higher in the GEN NTB group than in the control.

The OXTR protein level and OXTR mRNA expression levels were correlated significantly with the prosocial behavior of the GEN NTB children. This result was in line with the previous study (29,30,31), although, the neural mechanisms are not the subject of this current research.

The statistical analysis supported these findings as shown by bivariate and multivariate analyses that found significant relationships among variables. The multivariate analysis explained that the OXTR protein and OXTR mRNA independently had a significant correlation on the prosocial behavior of the GEN NTB children.

Current results were supported by previous research, which showed that a high OXTR gene was influenced by genetic, nutritional, and environmental factors.(30,31) To reduce the research bias, control was taken from the similar ethnic with equal socioeconomic properties. The influences of the OXTR protein and gene could be related to emotional and thinking patterns. A disturbance of the OXT system was closely related to Schizophrenia.(32) Other research results also showed that there was a significant association between the OXTR genes with the particular emotional aspect of empathy, and also correlated with individual social traits and behavior.(33-35)

In general, the results of this study were in accordance with the objectives of the GEN NTB program. Excellent prosocial behavior is developed from excellent parenting, environment, and nutrition in early life. Thus, individual behavior does not occur by the natural course only but also occurs as a result of a stimulus, both from internal and external individual factors. Authors relay that other factors may influence the development of prosocial behavior including nutritional intake and the history of hospitalization as well as chronic infection such as tuberculosis.(36,37) This study use the non-prospective design and not all factors that may influence prosocial behavior were observed, therefore future research supposed to be delivered with better design and control of other confounding factors.

Conclusion

The OXTR protein level and OXTR mRNA expression levels of the GEN NTB children were significantly higher compared to the control, and was correlated with prosocial behavior. This means that the social innovation of the GEN NTB program could be adopted as one of the programs to lift up the quality of human resources in early life.

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Authors Contribution

WI conducted the field survey and performed the data analysis. MH aided in the laboratory tests. SA, MH, SS supervised WI in the doctorate program. WI and FRA drafted and wrote the manuscript, while FRA and HK edited it. SA, MH, SS, and HK gave critical review for the manuscript. All authors read and approved the final manuscript.
References


34. Li J, Zhao Y, Li R, Broster LS, Zhou C, Yang S. Association of...

