Research Article

Calsium Supplementation with Rasbora sp. to Prevent Loss of Bone Mineral Density during Gonadotropin Releasing Hormone Agonist Long-term Treatment

Suplementasi Kalsium dengan Ikan Seluang (Rasbora sp.) untuk Pencegahan Kehilangan Kepadatan Mineral Tulang selama Penggunaan Jangka Panjang Agonis Gonadotropin-Releasing Hormone

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Abstract

Objective: To observe the benefit of calcium supplementation overcoming the hypoestrogenic effect of *Gonadotropin-releasing hormone* (GnRH) agonist, using a standardized regimen and *Rasbora sp.* (local: Seluang fish) as a natural source of calcium.

Methods: This study included 24 reproductive-aged (15-49 years old) females on subcutaneous agonist GnRH leuprolide acetate 11,25 mcg regimen. Bone mineral density was measured twice within three months, before and after the first agonist GnRH treatment. Subjects were classified into three groups of daily supplementations: placebo containing Saccharum lactis, ground powder of 500 mg of calcium, or ground powder of dried Rasbora sp containing 500 mg of calcium. Measurement pre- and post-supplementation were performed using the bone *quality index* with Osteosys of Sonost 3000.

Results: Following three months of observation, subjects receiving placebo had a decreased bone mineral density of -22,7201 compared to those who received 500 mg calcium supplementation and fish powder with a calcium content of 500 mg (-4,4570 and -3,3634, respectively). The homogeneity test showed a significance level of 0.031, and ANOVA resulted in a significant difference between the three classes. *Post Hoc* showed a significant difference between calcium lactate supplementation 18,26 + 3,20 (p = 0.001) and Seluang fish powder 19,36 + 3,20 (p = 0.000). Both forms of calcium lactate and fish powder supplementation had no significant differences.

Conclusion: Both calcium supplementations of calcium lactate powder and natural resources help maintain bone mineral density during GnRH agonist treatment. Seluang fish (Rasbora sp.), which is abundant in Indonesia and commonly consumed by the Indonesians, especially in South Borneo, has a similar potency to pharmaceutical 500 mg calcium lactate products. Fishery product contains other beneficial trace elements, such as 84 mg of calcium (Ca), 6,81 % of magnesium (Mg), 13,4 mg of iron (Fe), and 3.97 % of zinc (Zn).

Keywords: bone mineral density, calcium supplementation, GnRH agonist, *rasbora Sp.*

Abstrak

Tujuan: *Untuk mengetahui manfaat suplementasi kalsium dalam mengatasi efek hipoestrogenik oleh GnRH agonis menggunakan sediaan kalsium terstandar dan Rasbora sp. (lokal: Ikan Seluang) sebagai sumber kalsium alami.*

Metode: *Penelitian ini melibatkan 24 perempuan berusia reproduktif 15-49 tahun yang menjalani terapi GnRH agonis dengan regimen Leuprolide 11,25 mcg subkutan. Pengukuran terhadap densitas massa tulang dilakukan dua kali berselang 3 bulan sebelum dan sesudah pemberian regimen. Dalam waktu pengamatan, subjek dibagi secara acak dalam tiga kelas masing-masing berisi 8 orang yang mendapatkan suplementasi harian berisi salah satu dari plasebo (saccharum lactis), gerusan tablet 500 mg kalsium, dan tepung ikan seluang (Rasbora sp) dengan kandungan setara 500 mg kalsium. Hasil pengukuran sebelum dan sesudah pemberian regimen dihitung menggunakan indeks kualitas tulang dengan alat Sonost 3000 dari Osteosys.*

Hasil: *Perempuan pengguna GnRH agonis yang menerima plasebo mengalami penurunan kepadatan densitas tulang sebesar -22,7201 dibandingkan dengan mereka yang mendapatkan suplementasi 500 mg kalsium tablet dan tepung ikan dengan kalsium setara 500 mg, masing – masing -4,4570 dan -3,3634 setelah 3 bulan. Uji homogenitas menemukan signifikansi sebesar 0.031 dan ANOVA menunjukkan perbedaan bermakna antara ke tiga kelas tersebut yakni p=0.000. Uji Post Hoc menemukan perbedaan terjadi pada suplementasi kalsium tablet dibandingkan dengan placebo sebesar 18,26313 (p = 0.001) dan pengguna suplementasi tepung ikan seluang (Rasbora Sp.) sebesar 19,35675 (p = 0.000). Sedangkan suplementasi kalsium tablet dengan tepung ikan yang setara 500 mg, tidak terdapat perbedaan bermakna 1,09362 (p = 0.948).*

Kesimpulan: *Suplementasi kalsium baik dalam bentuk sediaan tablet maupun bahan alami membantu menjaga kepadatan massa tulang selama pemberian GnRH. Seluang (Rasbora sp.) yang melimpah dan dikonsumsi luas oleh masyarakat Indonesia khususnya di Kalimantan Selatan, memiliki potensi sama baiknya dengan produk farmasi berupa kalsium laktat setara 500 mg. Produk ikan, mengandung trace element lain yang baik untuk tubuh, selain 8.4 mg kalsium, terdapat juga 6.81 mg Magnesium (Mg), 1,339 mg Besi (Fe), dan 3.97 mg Zinc (Zn).*

Kata kunci: *GnRH Agonis, kepadatan massa tulang, Rasbora Sp., suplementasi kalsium.*

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INTRODUCTION

Long-term GnRH agonist therapy is used in several medical conditions related to both sex and growth hormone abnormalities. The pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are stimulated by GnRH agonist, especially as leuprolide acetate, thus increasing steroidogenesis of ovaries and testes and resulting in increased female estrogen production.[1] The hypoestrogenic condition could be a double-blade sword; while helping reduce the size of the benign gynecologic mass, including endometriotic cyst, due to its ability to decrease growth factor,[2] it could decrease the density of bone as it reduces proliferation and causes more prominent osteoclast activity than osteoblast.[3]

Bone mineral density starts to decrease when GnRH is suppressed and causes osteoclast to be more active, and residual loss of media percentage is highest after 12 months. Add-back therapy using calcium supplementation seems to reduce the rate of BMD reduction significantly.[4]

Quantitative ultrasound (QUS) has gained popularity as a technique to assess bone health since its introduction in 1984. It is highly accessible due to its portability, low cost, and simplicity of handling. In addition, no ionizing radiation is produced by QUS compared to dual-X-ray absorptiometry (DXA).[5] For precision of measurement, there isn't any difference in assessing bone mineral density and predicting osteoporosis.[6]

Supplementation of calcium during GnRH agonist therapy was observed and advised to prevent loss of bone mineralization.[7] Rasbora sp. is mainly found in the deep river around Nusantara (Indonesia's historical area),[8] whereas it is endemic, especially in the riverside plain on several main islands of Nusantara.[9] The species is considered to be the main source of protein or trace elements for indigenous people, who consume it as a daily diet.[10] Rasbora sp. is considered a genus of tiny fish of Cyprinidae that inhabit freshwater in South and Southeast Asia. They are small, with lengths ranging from 10 to 17 cm with many dark horizontal stripes.[11] The Indigenous name for this fish is Seluang, which has been believed to be a good source of calcium and protein for a long time.[12]

Figure 1. Silver rasbora or *Rasbora argryotaenia* (Local: Seluang fish).

METHODS

Subjects included females of reproductive age (15-49 years old) who presented to the gynecologic outpatient clinic of Ulin District Hospital in Banjarmasin, South Borneo, and were treated with GnRH agonist under any medical reason. The participants were selected using inclusion and exclusion criteria. Counseling and informed consent were obtained after detailed explanations to the patient and guardians.

Data included evaluation of age, blood pressure, and body mass index. During the study, patients were not restricted to any food but were randomly checked for a full-week food diary twice during the study. Later, participants were distributed into one of three groups. All study procedures were carried out according to the Human Research Ethics Committee from Ulin District Hospital.

A randomized controlled trial was applied to this single-centre, randomized, double-blinded, single-dose study. Data of bone quantity index (stiffness index) was examined in subjects before and after treatment using supplementation of either calcium or placebo.

Either subject and regiment were numbered and randomly distributed one sample for one subject. The supplementation regimen was classified into three classes consisting of the same number of samples: Saccharum lactis or fructose as placebo, ground powder of 500 mg lactate calcium, and ground powder of Seluang fish (Rasbora sp.) containing 500 mg calcium in equivalent, extracted and quantified by Certified Biochemistry Laboratory of Lambung Mangkurat University in Banjarbaru, South Borneo.

Every sample was packed into a capsule with the same size, colour, and weight. Each subject takes a daily capsule, which could consist of the following: placebo, ground powder of 500 mg lactate calcium, or ground powder of Seluang fish containing 500 mg calcium in equivalent for three months. The fish is processed into powder within three steps; the fish is minced together without separating flesh and bones, and the mixture is then steamed within 15 minutes and pressed to extract the water. Later on, it is dried under sun rays for 1 day, and it is further blended and ground into powder.[13]

Then, extracts were verified in a biochemistry laboratory for the calcium portion in the sample. Based on the sample in 065/UN.8.1.17.2.2/ PP/2019 fishery product, this sample contains trace elements, which are beneficial (consisting of 84 mg of calcium (Ca), 6.81 %mass of magnesium (Mg), 13,4 mg of iron (Fe), and 3.97 %mass of zinc (Zn) for every 100 mg powder of extract).

The bone quality index (BQI) was evaluated using Osteosys of Sonost 3000 before any treatment was given. Evaluation of the second attempt to retrieve bone mineral density data was done after three months of supplementation following the first subcutaneous injection of GnRH agonist depot consisting of 11,25 mcg Leuprolide acetate that lasted for three months. Patients' complaints were solved based on symptoms and documented.

The bone mineral density (BMD) is expressed in the stiffness index or called the bone quantity index. We used the Student's t-test or one-way ANOVA (analysis of variance) to determine the difference between BMD before and after GnRH agonist and supplementations. We subsequently performed posthoc Duncan's multiple range test ($p<0.05$ is considered significant). We used Leven's test to determine the homogeneity of variance for ANOVA and t-test. Statistical Product and Service Solutions (SPSS) 25th version released by IBM was used for all statistical analyses. The odd ratio is analyzed for each group to find the quantitative difference between classes.

RESULTS

This study included twenty-four subjects who completed the randomized, single-dose, closed-label, single-centre, oral supplementation study within 3 months. All characteristics data were presented in table 1. The result showed that age, parity, and BMI did not differ between groups. Hence, it shows that participants were fairly distributed.

Subjects receiving placebo had a decreased bone mineral density of -22,7201 compared to those who received 500 mg calcium supplementation and fish powder with a calcium content of 500 mg (-4,4570 and -3,3634, respectively).

Table 1. Basic Characteristics Data of Participants.

Characteristic (Mean ± SE)	Group 1 Placebo	Group 2 Oral pill (500 mg calcium)	Group 3 Ground seluang (500 mg calcium)	Significance *(P-value)*
Age (mean, years old)	38.00 ± 2.260	42.75 ± 1.461	38.13 ± 1.977	0.164
Parity (mean)	0.75 ± 0.313	0.75 ± 0.366	0.88 ± 0.398	0.961
0 (*n*)	4	4	4	
1 (*n*)	2	3	2	
> 2 (*n*)	2	1	2	
BMI (mean, kg/m^2)	26.83 ± 1.447	25.71 ± 1.652	27.66 ± 1.922	0.717
Random calcium intake (mg/day)	56.33 ± 3.402	53.21 ± 2.843	55.16 ± 3.221	0.653
BQI before therapy	76.75 ± 5.681	79.22 ± 4.210	82.19 ± 6.378	0.785
Change in BQI	- 22.72 ± 1.775	- 4.46 ± 2.967	- 3.36 ± 1.855	0.000

The stiffness index or bone quantity index showed a significant difference; therefore, the result is tested using ANOVA, homogeneity using Levene's test, and post hoc comparison between groups using the Bonferonni test.[14] Correlation in classes from paired T-Test was significant for each class showing that data were taken from the same sample, while changes were significant only in the placebo class, showing that paired data has significant changes in that group only.

Table 2. Differences in changes in BQI between groups

Group (Mean ± SD)	before therapy and supplementation (*p* of Normality)	after therapy and supplementation (*p* of Normality)	Changes (*p* of Homogeneity)	Paired T Test *p1* of Correlation test *p2* of Changes test
Placebo	76.75 ± 16.07 (0.918)[a]	54.03 ± 20.33 (0.670)[a]	22.72 ± 5.02 (0.667)[b]	*p1* = 0.000[c] *p2* = 0.000[d]
Oral pill (500 mg calcium)	79.22 ± 11.91 (0.068)[a]	74.76 ± 7.04 (0.998)[a]	4.46 ± 13.95 (0.199)[b]	*p1* = 0.043[c] *p2* = 0.117[d]
Ground seluang (500 mg calcium)	82.19 ± 18.04 (0.231)[a]	78.83 ± 19.01 (0.465)[a]	3.36 ± 5.24 (0.913)[b]	*p1* = 0.000[c] *p2* = 0.113[d]

Note: [a] using Saphiro Wilk, [b] using Levene, [c] and [d] using correlation and changes in paired T-test

The homogeneity test showed a p-value of 0.031. The ANOVA showed a significant difference between the three classes. Mean and SE differences in stiffness index or bone quantity index were presented in figure 2.

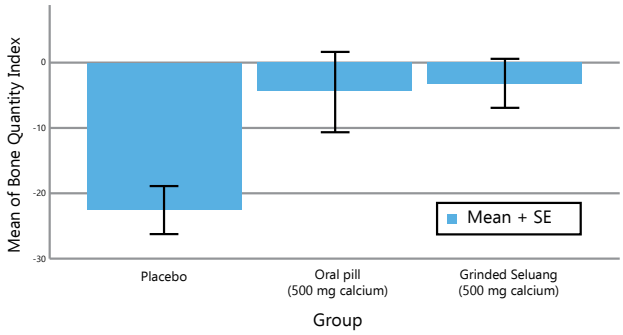

Figure 2. Mean and Standard Error of difference in bone quantity index (BQI) from each group of calcium supplementation.

Data were taken before and after receiving GnRH agonist for 3 month. Calcium supplementation lowered loss in BQI during GnRH agonist therapy. Compared to placebo, *post hoc* test using Bonferonni formula showed a calcium lactate supplementation of 18.26 + 3.20 (p = 0.001) and ground Seluang powder of 19.36 + 3.20 (p = 0.000). Calcium lactate supplementation and ground Seluang powder did not have a significant difference.

DISCUSSION

Long studied by Treber et al. of bone calcium loss during GnRH agonist therapy,[15] current guidelines recommend supplementation of calcium to prevent the further possibility of osteoporosis due to loss of bone calcium.[16] Calcium supplementation could potentially provide hormonal add-back therapy to prevent blood loss. But several conditions that need hypoestrogenic conditions are not suited to this add-back therapy.4 GnRH agonist therapy in dysregulated puberty, either late or precocious, and follicle stimulation could have an advantage over non-calcium supplementation.[17]

Seluang was recommended as calcium supplementation since it contains abundant organic calcium.[18] Result from other studies[19,20] showed that 100 grams of Seluang contain 80 mg calcium, 224 mg phosphorus, and 4.7 mg iron. Organic calcium comes with other trace elements that could enhance or benefit participants.

CONCLUSION

Calcium supplementation helps to maintain bone mineral density while patients are in GnRH agonist treatment. A daily dose of 500 mg whether in calcium lactate or organic calcium extracted from Seluang fish (Rasbora sp.), the primary fish group particularly found in South Borneo, has a similar potency to 500 mg pharmaceutical calcium lactate. Local people can benefit from daily intake of seluang fish as an economic resource of calcium while taking medication. For recommendation, further studies of multi-center calcium supplementation using a prepared regimen or local resources are necessary to assess ethnic-related calcium metabolism—while using calcium supplementation when undergoing GnRH agonist medication.

REFERENCES

1. Seo JW, Lee DY, Kim SE, Yoon BK, Choi DS. Comparison of long-term use of combined oral contraceptive after gonadotropin-releasing hormone agonist plus add-back therapy versus dienogest to prevent recurrence of ovarian endometrioma after surgery. Eur J Obstet Gynecol and Reprod Biol. 2019;236:53-7.
2. Rafique S, Decherney AH. Medical Management of Endometriosis. Clin Obstet Gynecol. 2017;60(3):485-96.
3. Panday K, Gona A, Humphrey MB. Medication-induced osteoporosis: screening and treatment strategies. Ther Adv Musculoskelet Dis. 2014;6(5):185-202.
4. Osuga Y, Torng PL, Jeng CJ. Clinical studies investigating the use of leuprorelin in Asian women with endometriosis: a review. J Obstet Gynaecol. 2019;1:1-7

5. Chin KY, Ima-Nirwana S. Calcaneal quantitative ultrasound as a determinant of bone health status: what properties of bone does it reflect?. Int J Med Sci. 2013;10(12):1778-83.
6. Newcomer, Beth R. Comparison of Two Bone Mineral Density Pre-Screening Tools: QUS and RA, to the DXA. Electronic Theses and Dissertations. 2010:106.
7. Sandra BA, Francisco AP, Rosângela AV, et al. Bone mineral density and body composition in girls with idiopathic central precocious puberty before and after treatment with a gonadotropin-releasing hormone agonist. Clin. 2012;67(6):591-6.
8. Alpiyah N, Purnengsih I. Karakter Gajah Mada: Simbol Kejayaan Majapahit. Visual Heritage. Jur Kreasi Seni dan Budaya. 2019;1(2):1-3
9. Dina R, Samir O, Lukman, Haryani GS, Nasution SH. Fish and fisheries of Bada (Rasbora spp.) in Lake Maninjau, West Sumatra. IOP Conf. Ser. Earth Environ. Sci. 2018:306
10. Partan RU, Salim E, Hidayat R. The Potential Role of Seluang Fish Oil (Rasbora Spp) in Improving Calcium, Zinc and Vitamin D3 Deficiencies. Int J Oceans Oceanography. 2017;11(2): 285-91.
11. Muchlisin ZA. Morphometric Variations of Rasbora Group (Pisces: Cyprinidae) in Lake Laut Tawar, Aceh Province, Indonesia, Based on Truss Character Analysis. HAYATI. J Biol Sci. 2013;20(3):138-43.
12. Triawanti, Yunanto A, Sanyoto D. The potential of seluang fish (Rasbora spp.) to prevent stunting: The effect on the bone growth of Rattus norvegicus. Proceedings of the 4th BIBMC (Bandung International Biomolecular Medicine Conference) 2016 and the 2nd ACMM (Asean Congress on Medical Biotechnology and Molecular Biosciences). 2017
13. Napitu N. Pembuatan Ekstrak Dan Tepung Ikan Gabus Sebagai Substitusi Pengolahan Biskuit Untuk Menaikkan Kadar Albumin Pada Balita Gizi Buruk. Jurnal Pengabdian Kepada Masyarakat. 2016;16(60):42-6.
14. Davison ML, Sharma AR. ANOVA and ANCOVA of pre- and post-test, ordinal data. Psychometrika. 1994;59:593–600.
15. Waibel-Treber S, Minne HW, Scharla SH, Bremen T, Ziegler R, Leyendecker G. Reversible bone loss in women treated with GnRH-agonists for endometriosis and uterine leiomyoma. Hum Reprod. 1989;4(4):384-8.
16. Carrie Armstrong. ACOG Updates Guideline on Diagnosis and Treatment of Endometriosis. Am Fam Physician. 2011;83(1):84-5.
17. Kaya A, Cayir A, Turan MI, Ozkan B. An Examination of the Effects of Leuprolide Acetate Used in the Treatment of Central Precocious Puberty on Bone Mineral Density and 25-Hydroxy Vitamin D. West Ind Med J. 2015;64(2):104-7.
18. Putri AS, Kusfriyadi MK. Pengaruh Substitusi Tepung Selpis (Seluang Dan Pisang) Terhadap Kadar Protein, Kalsium, Daya Terima Dan Mutu Organoleptik. Jur Ris. 2020;1(1):1-3.
19. Triawanti, Yunanto A, Sanyoto DD, Nur'amin HW. Nutritional Status Improvement in Malnourished Rat (Rattus norvegicus) after Seluang Fish (Rasbora spp.) Treatment. Curr Res Nutr Food Sci. 2018;6(1):127-34.
20. Triawanti, Noor MS, Nur'amin HW, Sanyoto DD. The seluang fish (Rasbora spp.) diet to improve neurotoxicity of endosulfan-induced intrauterine pup's brain through of oxidative mechanism. ClinNutr Experimental. 2019;28:74-82.