Short tandem repeat near hypoxia response element (HRE) instead of HRE genetic variants in promoter calcitonin receptor-like receptor (CRLR) gene as risk factor in severe preeclampsia: a preliminary study

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Abstract
Objective: Calcitonin receptor-like receptor (CRLR) regulates vasoconstriction and dilatation; the expression increases during hypoxia via activation of hypoxia response element (HRE) in CRLR gene promoter region. Variant in HRE, as well short tandem repeat (STR) variants near HRE in CRLR alters the gene expression. This study focused on a case–control study to investigate the expression of genetic typing CRLR promoter variant in pregnant women with severe preeclampsia and normal pregnancies, we also tried to describe interesting findings of the genetic expression in anemic patients in the severe preeclampsia group. Our aimed to observe the correlation of CRLR gene promoter variant and anemia in severe preeclampsia.

Results: There was no nucleotide variant in HRE; CACA box prior to HRE varied in length (15–24); CACA box with length > 20 was used as cut off point. Hb was lower in CACA box length ≥ 21 (10.33 ± 1.57) vs. < 21 (11.01 ± 1.67; p = 0.391). CACA box polymorphism and anemia were correlated in severe preeclampsia (p = 0.005) OR 0.038 (CI 0.003–0.544); not in normal (p = 0.069).

Keywords: Preeclampsia, CRLR, CA repeat, HRE, CACA box polymorph, Gene promoter

Introduction
Hypertension and anemia are common disorders in pregnancy. Preeclampsia is one of hypertensive disorders and anemia in preeclampsia patient can be related to HELLP syndrome or isolated pathological condition, in case of simultaneous occurrence, the risk of maternal mortality and morbidity are higher. Preeclampsia and anemia affect mother and baby outcomes due to chronic hypoxia [1–4]. Adrenomedullin signaling plays important role in vessel dilatation and constriction in pregnancy; depends on the interaction of calcitonin gene related peptide (CGRP), calcitonin receptor-like receptor (CRLR) and receptor activity modifying protein (RAMP). CRLR expression in preeclampsia reported to be altered and increased in hypoxia condition including hypertension and anemia, although in preeclampsia some studies said otherwise [5–9]. CRLR encoded by CRLR, located in 2q32.1. CACA box length, a STR in CRLR and HRE structure close to transcription initiation site, play role in protein expression as in other...
gene and affected phenotype. In other gene, CACA box length, HRE genetic variant alter protein expression and related to many pathologic condition. CACA box with (AC) 17–39 repeat in the promoter region of the HO-1 gene, polymorphisms of which is associated with cardiovascular diseases, Parkinson’s, cancer and preeclampsia [10, 11]. HRE nucleotide variants in several genes affected gene expression via hypoxia induced factor (HIF) binding [12, 13]. In preeclampsia, chronic hypoxia can occur. Anemia in pregnancy can also lead to chronic hypoxia. Anemia and Preeclampsia affected mothers and baby outcomes, when it happened simultaneously related to higher maternal mortality and morbidity. There have been several studies conducted to investigate CACA box length and HRE genetic variants in CRLR gene promoter in patients with severe preeclampsia, but studies on the review of genetic expression of severe preeclampsia by looking at hemoglobin levels in severe preeclampsia patients have never been done [1–4].

Main text

Material and methods

Study subject and sampling collecting

This study obtained permission from the Faculty of Medicine and Health Sciences, Jambi University ethical research committee. We conducted case–control study involving 40 patients, separated into two groups. Case group (20 patients) with severe preeclampsia and control group (20 patients) with normal pregnancy. The study participants gave birth in Raden Mattaher Hospital, Jambi, Indonesia. The inclusion criteria for severe preeclampsia based on systolic blood pressure ≥ 160 mm Hg or diastolic blood pressure ≥ 110 mm Hg with or without proteinuria after 20 weeks of gestation. Hemoglobin level, demographic data, and obstetric history were ascertained based on hospital records and reconfirmed to the patients when placental tissue and maternal blood samples were taken. The exclusion criteria are multiple pregnancies, premature labor, previous history of chronic diseases and acute inflammatory diseases. Peripheral venous blood collected from mother before gave birth in an etilenadiminetetraasetat acid (EDTA)-coated tube for DNA and PCR. PCR and DNA sequencing used for genotyping placenta tissue and maternal blood in both group. We investigate hypoxia-induced factor (HIF) to hypoxia response element (HRE) in CRLR gene promoter region. Variant in HRE altered HIF, as well short tandem repeat (STR) variants near HRE in CRLR. CACA box is STR, genetic variants determined transcriptional complexes located outside the STRs related to difference in distance to transcription start site.

Isolation of DNA, PCR and genotyping

DNA was extraction from peripheral blood sample solid phase method by Chelex-100 resin Catalogue No 7610010, purity DNA extraction determined with Spectrophotometry. Polymerase Chain Reaction (PCR) used to amplified promoter CRLR genome segment suspected, forward primer 5’ GAGGAAACAGACACACCATTA 3’ and reverse primer 5’ GCTGGCTTTCACCTTGACTG 3’. The PCR product was 304 base pairs, and run in electrophoresis. Denaturation temperature was 94°C for 50 s, annealing temperature was 59°C for 50 s for 35 cycle and extension in 70°C for 10 s. Genotyping of PCR product used Sanger Sequencing method, the sequence data was analyzed by Bio-edit VII software by matching the data with reference sequence from Gene Bank. Gene ID 10203 was used as reference sequences.

Statistical analysis

Participant characteristic analyzed with t-test for quantitative- and chi square for qualitative-data. Cut off point CACA box length polymorphism based on mean value of participants. Correlation of CACA box length and preeclampsia analyzed with chi square. Subgroup analysis was perform to find conditions related to preeclampsia with CACA box genetic variant. A p < 0.05 was considered significant. Statistical analyses were performed with IBM SPSS statistic 23 software.

Results

Baseline characteristic study participants

We assessed 40 pregnancy women who gave birth at Raden Mattaher Hospital, Jambi, Indonesia within 2017. All study participants were full term pregnancy at delivery, Malayan ethic resided at Jambi Province and had been signed informed consent. We modeled the baseline characteristic of study participants. In model of severe-vs. normal-pregnancy, age distribution was older, leucocytes count was lower, thrombocytes count slightly higher, anemia was lower in severe pregnancy group but statistically insignificant. In severe preeclampsia group we found higher systolic blood pressure, higher diastolic blood pressure, higher hemoglobin level, lower anemia frequency, higher pathologic proteinuria and lower baby birth weight, all of the variables were statistically significant (Table 1).

Grouping of patients based on hemoglobin level (cut off point for hemoglobin level was 11 mg/dl). In this grouping, systolic blood pressure was higher, diastolic blood pressure was higher, hemoglobin level was higher, leucocytes count was lower, thrombocytes count was lower, pathologic proteinuria was higher and baby weight was lower in normal hemoglobin level, all variables were not
statistically significant. We also found that older age and higher severe preeclampsia frequency in normal hemo-
globin level group, both statistic significant (Table 1).

**Genotyping genetic variants**

HRE in CRLR promoter is located downstream just after CACA box and in close proximity to transcriptional start site. HRE is gene promoter sequences (5’-CACGC-3’) where HIF bind and effected gene expression; no polymor-
phism of nucleotide sequences found in our population (Additional file 1). Alteration of transcription factor binding in previous study related to STR genetic variants and we found STR as CACA box genetic variant near HRE sequences. Based on genotyping in our study population we found that CACA box length variant of nucleotide sequences pattern.

Grouping of patients based on CACA box length found that there was no statistically difference between all vari-
ables. Hemoglobin level was lower (10.41 ± 1.67) in polymorphic group than in normal variant (10.88 ± 1.63; p = 0.391). Anemia frequency was higher in polymorphic group (64.7%) than in normal variant (43.5%) and not statistically significant (p = 0.313). Subgroup analysis showed that CRLR polymorphism was a risk factor for anemia with OR 0.038 (CI 0.038–0.544) in severe preeclampsia, showed in Table 2. Addition of CACA box analysis, our study participant genotyping showed no nucleotide sequences variances. It is indicated that HIF binding in CRLR gene promoter influenced by genetic variants of CACA box length instead of HRE DNA sequences pattern.

**Discussion**

CRLR codes CRLR protein, a GCPRs family. CRLR is receptor for CGRP ligand; activated by protein called RAMPs and recognized as integral component of the adrenomedullin signaling system that regulated vasodil-
atation, vessel permeability, inhibition endothelial cell apoptosis and promotion of angiogenesis. Adrenomedul-
lin signaling play important role in preeclampsia patho-
physiology [5, 7–9]. Preeclampsia, a hypertensive disorder spectrum caused by dysregulation of vasocon-
striction and vasodilatation. Anemia is one of the hema-

tologic problems in pregnancy because of the physiologic changes in pregnancy. Anemia in preeclampsia can be part of preeclampsia or a separated pathologic condition,
both related to chronic hypoxia and increased maternal and perinatal mortality [1–3].

Genetic variant of CRLR alter its expression, CRLR alteration found in preeclampsia and anemia related to hypoxic condition. HRE in other gene regulated protein transcription through binding with HIF transcription factor. HRE gene variant associated to compensation mechanism in hypoxia as well in anemia [12, 13]. There was no HRE nucleotide mutation in our population. It might indicate that HIF binding in CRLR gene promoter influenced by genetic variants of CACA box length instead of HRE DNA sequence motives.

Regulation of transcription factor in hypoxia related to other DNA sequence motives like CACA box length upstream 5′ from TSS. CACA box is STR that consist AC repeat difference and predominant in some gene promoter such as CRLR. STR does not bind transcription factor but determine other nucleotide sequences to bind transcription factor. The difference of CACA box length relates to difference of distance to TSS. It is related to difference of transcription rate and might influence gene expression. Higher protein expression found in shorter CACA box length in some gene [10, 11]. We showed lower hemoglobin level in polymorphic group. In addition, when we stratified severe preeclampsia into two groups anemia and non-anemia, we found polymorphic more frequent in the anemia vs. non-anemia group. We suggest polymorphic group who had longer CACA box length and correlated with lower CRLR expression might compensated mechanism of hypoxia related to anemia and preeclampsia. They maintain vasoconstriction in condition of lower hemoglobin level, although the mechanism correlated with these two were not clear yet.
Conclusion
There was no HRE genetic variants in our study population. Anemia in severe preeclampsia was more frequent in polymorphic group, who had longer CACA box length. Larger sample are needed to see correlation of genetic variant of CRLR gen promoter with severe preeclampsia and anemia.

Limitation of the study
The limitation of this preliminary study was small sample size; further sample with larger sample was needed to demonstrate association between promoter CRLR gene variants with severe preeclampsia, anemia and other hypoxia condition. Further study is needed to demonstrate others DNA sequence motives in CRLR gene regulated CRLR receptor expression or other signaling that more dominant than adrenomedullin signaling in hypoxia condition.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13104-020-05437-z.

Additional file 1: Figure S1. CRLR Gene Sequencing. Red box showed sequencing analyzed of CACA box on our sample and blue box showed HRE sequences.

Abbreviations
HRE: Hypoxia response element; HIF: Hypoxia induced factor (HIF); CRLR: Calcitonin receptor-like receptor; STR: Short tandem repeat; HELLP: Hemolysis, elevated liver enzyme, and low platelet count syndrome; STR: Short tandem repeat, CGRP: Calcitonin gene related peptide; RAMP: Receptor activity modifying protein; EDTA: Ethylene diamine tetraacetic acid; PCR: Polymerase chain reaction.

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Authors’ contributions
H and ADF had examined, treated, observed and followed up the subject of this study. AS, Y and APE had read and interpreted the results. RNE and AF had examined, treated, observed and followed up the subject of this study. AS, Y and APE had read and interpreted the results. RNE and AF had examined, treated, observed and followed up the subject of this study. AS, Y and APE had read and interpreted the results. RNE and AF had examined, treated, observed and followed up the subject of this study. AS, Y and APE had read and interpreted the results. RNE and AF had examined, treated, observed and followed up the subject of this study. AS, Y and APE had read and interpreted the results. RNE and AF had examined, treated, observed and followed up the subject of this study.

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Availability of data and materials
Authors declare that the data will not be shared since they are patients’ confidentiality.

Ethics approval and consent to participate
This study protocol was approved by Faculty of Medicine Ethics Committee Review Board, University of Jambi; all participants gave written informed consent and agree to participate.

Consent to publish
Not applicable.

Competing interests
Authors have declared that no competing interests exist.

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References
1. World Health Organization. Recommendation for prevention and treatment of pre-eclampsia and eclampsia. Geneva: WHO press; 2011.
2. Cunningham FG, Leveno KJ, Bloom SL, et al. William obstetrics. 24th ed. New York: McGraw Hill Education; 2014.
3. American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists Task Force on Hypertension in Pregnancy. Obstet Gynecol. 2013;122(5):1122–31. doi: https://doi.org/10.1097/01.AOG.0000457382.03963.88.
4. Robillard PY, Dekker G, Chauot G, Sciscia M, Iacobelli S, Hulsey TC. Historical evolution of ideas on eclampsia/preeclampsia: A proposed optimistic view of preeclampsia. J Reprod Immunol. 2017;125:72–7. https://doi.org/10.1016/j.jri.2017.09.006.
5. McGuane J, Conrad K. GPCRs as potential therapeutic targets in preeclampsia. Drug Discov Today Dis Models. 2012;9(3):e119–27. https://doi.org/10.1016/j.ddmod.2012.05.001.
6. Ariza AC, Bobadilla N, Diaz L, Avila E, Larrea F, Halhali A. Placental gene expression of calcitonin gene-related peptide and nitric oxide synthases in preeclampsia: effects of magnesium sulfate. Magnes Res. 2009;22(1):44–9.
7. Dong YL, Green KE, Vegiragu S, Hanksins GD, Martin E, Chauhan M, Thota C, Yallampalli C. Evidence for decreased calcitonin gene-related peptide (CGRP) receptors and compromised responsiveness to CGRP of feto-placental vessels in preeclamptic pregnancies. J Clin Endocrinol Metab. 2005;90(4):2336–43. https://doi.org/10.1210/jc.2004-1481.
8. Nikitenco LL, Smith DM, Bicknell R, Rees MC. Transcriptional regulation of the CRLR gene in human microvascular endothelial cells by hypoxia. FASEB J. 2003;17(11):1499–501.
9. Lernhart PM, Caron KM. Adrenomedullin and pregnancy: perspectives from animal models to humans. Trends Endocrinol Metab. 2012;23(10):524–32. https://doi.org/10.1016/j.tem.2012.02.007.
10. Sawaya S, Bagshaw A, Buschiazzo E, Kumar P, Chowdhury S, Black MA, Gemmell N. Microsatellite tandem repeats are abundant in human promoters and are associated with regulatory elements. PLoS ONE. 2013;8(2):e54710. https://doi.org/10.1371/journal.pone.0054710.
11. Bagshaw ATM. Functional Mechanisms of Microsatellite DNA in Eukaryotic Genomes. Genome Biol Evol. 2017;9(9):2482–43. https://doi.org/10.1093/gbe/evx164.
12. Sahvi A, Thanabalu T. Expression of N-WASP is regulated by HIF-1α through the hypoxia response element in the N-WASP promoter. Biochem Biophys Rep. 2016;9:13–21. https://doi.org/10.1016/j.bbrep.2016.10.010.
13. Slenc M, Kuné T. Transcription factor HIF-1A: downstream targets, associated pathways, polymorphic hypoxia response element (HRE) sites, and initiative for standardization of reporting in scientific literature. Tumour Biol. 2016;37(11):14851–61. https://doi.org/10.1007/s13277-016-5331-4.

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