Original Article

H6PD Gene Polymorphisms (R453Q and D151A) and Polycystic Ovary Syndrome: A Case-Control Study in A Population of Iranian Kurdish Women

Rozita Naseri, M.D.[1], Yosra Alimoradi, M.D.[1], Maryam Sohrabi, M.Sc.[2], Mostafa Cheraghian Fard, M.Sc.[2], Elahe Barzingarosi, M.D.[1], Amir Abdolmaleki, Ph.D.[2], Cyrus Jalili, Ph.D.[2*]

1. Internal Medicine Department, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran
2. Department of Anatomical Sciences, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran

Abstract

Background: Polycystic ovary syndrome (PCOS) is known as the most common endocrine and metabolic disorder in the reproductive-age women. Due to the effects of PCOS on the physical and mental health, the investigation of the factors affecting the development of PCOS is crucial. Hexose-6-phosphate dehydrogenase (H6PD) is a microsomal enzyme that catalyzes the first two reactions of the oxidative chain of the pentose phosphate pathway. The present study examined the polymorphisms of the *H6PD* gene (R453Q and D151A) in PCOS patients of Iranian Kurdish women.

Materials and Methods: In this case-control study, a total, of 200 female volunteers in two equal groups participated in our study. The PCOS patients were selected based on the Rotterdam diagnostic criteria. The association of *H6PD* gene polymorphisms, D151A and R453Q, with the development of PCOS were investigated. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used for genotyping. Statistical analysis was applied by the SPSS (version 16) software.

Results: Statistically significant lower frequencies of AA+AG genotype (37% vs. 55%, P=0.01) and A allele (22.5% vs. 34%, P=0.01) of R453Q were observed in the patients compared to the controls. In the case of D151A, no significant differences were observed in the frequency of genotypes and alleles between the two groups.

Conclusion: The findings of this study suggest that variants of H6PD R453Q affect the risk of PCOS.

Keywords: Polycystic Ovary Syndrome, Polymerase Chain Reaction, Polymorphism

Citation: Naseri R, Alimoradi Y, Sohrabi M, Cheraghian Fard M, Barzingarosi E, Abdolmaleki A, Jalili C. H6PD gene polymorphisms (R453Q and D151A) and polycystic ovary syndrome: a case-control study in a population of iranian kurdish women. Int J Fertil Steril. 2022; 16(3): 180-183. doi: 10.22074/IJFS.2021.141690.1050.
This open-access article has been published under the terms of the Creative Commons Attribution Non-Commercial 3.0 (CC BY-NC 3.0).

Introduction

Polycystic ovary syndrome (PCOS) is known as an endocrine disorder with oligomenorrhea, chronic anovulation, hyperandrogenemia, and polycystic ovary morphology (1). Although the findings of a review study of twins and familial segregation patterns provide convincing evidence for genetic etiology, there is no obvious Mendelian inheritance pattern of PCOS (2); it is widely accepted that multiple genetic and environmental factors and their complex interaction are involved in the PCOS occurrence (3). Multiple disorganizations related to this endocrine disorder can lead to various pathological conditions such as irregular menstruation, hyperandrogenemia, high luteinizing hormone/follicle-stimulating hormone (LH/FSH) ratio, infertility, obesity, insulin resistance, and ovulatory dysfunction (4-6). The etiology of this complex heterogeneous disease is still unknown which has attracted the attention of many scientists (7).

In this complex disorder, PCOS, the genetic and environmental factors are closely related to the insulin resistance that leads to steroidogenesis dysregulation. On the other hand, PCOS is mimicked by steroidogenesis defects that exist in hyperandrogenemia like congenital adrenal hyperplasia and cortisone reductase deficiency (CRD) which possess a monogenic base. Also, CRD results in the 11β-hydroxysteroid dehydrogenase type 1 enzyme (11β-HSD1) regeneration failure (8). CRD is seen in hyperandrogenic signs, including oligo-amenorrhea, hirsutism, and female infertility, as well as premature puberty in males (9). The similarities between the CRD and the PCOS phenotypes are focused on the *HSD11B1* and *H6PD* genes to describe the overloaded androgen and adrenal hyperandrogenism which are common in the PCOS phenotypes (10).

Hexose-6-phosphate dehydrogenase (*H6PD*) gene is located on chromosome 1p36.22, spans 37 kb, including 5 exons. It encodes H6PD protein, a microsomal enzyme that catalyzes the first two reactions of the oxidative chain of the pentose phosphate pathway (11-13). H6PD influences the activity of the 11β-HSD enzyme, which is involved in cortisol metabolism. Also, H6PD through the 11β-HSD en-

Revised: 28/November/2020, Accepted: 01/November/2021
*Corresponding Address: P.O.Box: 1565, Department of Anatomical Sciences, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran
Email: cjalili@yahoo.com

zyme can modulate insulin sensitivity in the adipose tissue (10). It has been reported that mutation in the *H6PD* gene is associated with the PCOS phenotype (4, 8).

Gene polymorphisms are common DNA sequence variations among populations that are important in the development of several hereditary diseases. There are contradictions in the role of two polymorphisms in the *H6PD* gene, namely D151A (rs4603401) and R453Q (rs6688832) in the PCOS phenotype (8, 10, 14, 15). Therefore, a case-control study was designed to investigate the association of these two polymorphisms with PCOS in a population of Kurdish women in the West of Iran.

Materials and Methods

Subjects

In this case-control study, 200 Kurdish women participated from the West of Iran. They are divided into two equal groups, PCOS patients and healthy controls. The healthy control group was matched by demographic characteristics in the patient group. The inclusion and exclusion criteria were based on the revised Rotterdam diagnostic criteria (16): briefly, i. Oligo or anovulation, ii. Sign of hyperandrogenism, iii. Polycystic ovaries. The presence of two items of Rotterdam diagnostic criteria is necessary to consider a definite PCOS. According to the Rotterdam diagnostic criteria, members of each group were verified by a gynecologist. This study was approved by the Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran (IR.KUMS.REC.1397.260). And also, the informed consent form approved by the KUMS Ethics Committee has been signed by all participants.

DNA extraction

Using the salting-out method, DNA was extracted from peripheral blood samples (17) and stored at -20°C until use. The quality and quantity of isolated DNA samples were evaluated through agarose gel electrophoresis and Nanodrop spectrophotometer (Thermo, USA).

Genotyping

Polymerase chain reaction (PCR) amplification was used to assess the variants of H6PD (R453Q and D151A) followed by the restriction fragment length polymorphism (RFLP) analysis. PCR primers for amplification of the region spanning the selected polymorphisms were designed using NCBI (https://www.ncbi.nlm.nih.gov/) and Oligo 7 software (Table 1). PCR was performed in a total volume of 25 µl containing 2.5 µL of 10X PCR buffer, 20 pmol of each forward and reverse primers (SinaClon BioScience Co., Iran), 1.5 mM $MgCl_2$, 0.2 mM dNTPs (SinaClon BioScience Co., Iran), 1 unit of Taq DNA polymerase (SinaClon BioScience Co., Iran) and about 100 ng of extracted DNA as a template. The thermal cycler program included an initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 40 seconds, annealing at 62°C (R453Q)/64°C (D151A) for 35 seconds, extension at 72°C for 40 seconds, and finally one cycle of extension at 72°C for 5 minutes. To perform RFLP, 10 µl of PCR products were digested with 2 units of MboII restriction enzyme (Thermo, USA) at 37°C for D151A and PstI restriction enzyme (Thermo, USA) at 37°C for R453Q, for 15 hours. Products of enzyme digestion were visualized after 2% agarose gel electrophoresis and staining with GelRed (Kawsarbiotech, Iran) under ultraviolet light (Figs.1, 2).

Table 1: The sequence of primers

NCBI rs#	SNP	Primer sequences (5´-3´)
rs4603401	D151A	F: agctgagccagtaccgccaac R: gctgatgctcacctgcttgccta
rs6688832	R453Q	F: actacgcctacagccctctgc R: ccaggaggccagcaagttctc

SNP; Single nucleotide polymorphism.

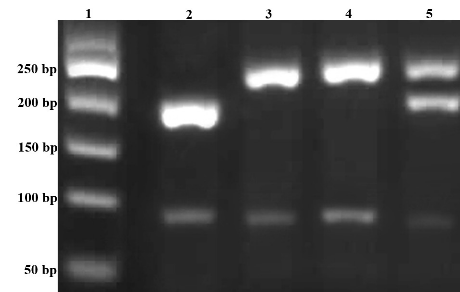

Fig.1: PCR-RFLP products of *H6PD* (D151A) on 2% agarose gel electrophoresis. 1; 50 bp DNA ladder, 2; Homozygote AA, 191 and 83 bp fragments, 3, 4; Homozygote CC, 223 and 83 bp fragments, 5; Heterozygote AC, 223, 191, and 83 bp fragments. PCR-RFLP; Polymerase chain reaction-restriction fragment length polymorphism.

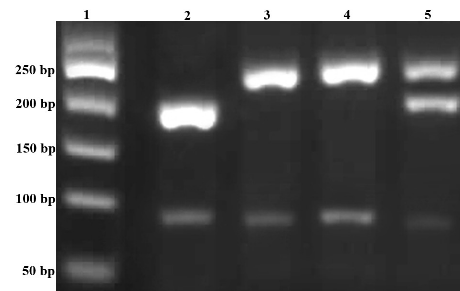

Fig.2: PCR-RFLP products of *H6PD* (R453Q) on 2% agarose gel electrophoresis. 1; 50 bp DNA ladder, 2, 4; Homozygote GG, 113 bp fragment, 3; Homozygote AA, 93 bp fragment, 5; Heterozygote AG, 113 and 93 bp fragments, and PCR-RFLP; Polymerase chain reaction-restriction fragment length polymorphism.

Statistical analysis

A statistical package for social sciences (IBM SPSS vs. 16, Chicago II, USA) was used for statistical analysis. Pearson's chi-square (χ^2) test analyzed genetic distribution variations between two groups. The odds ratios (OR), with 95% confidence intervals (95% CI), were measured with an unconditional logistic regression model.

Results

The mean age of the patient group was 27.09 ± 5.19 that in comparison with the control group (28.34 ± 4.58)

showed no significant difference (P=0.073). In terms of body mass index (BMI), a significant difference was observed, (29.14 ± 2.68 vs. 23.05 ± 2.48 for patient and control groups, respectively (P=0.001).

The frequency of *H6PD* genotypes in patients with PCOS was in Hardy-Weinberg equilibrium (P=0.27 for D151A, P=0.09 for R453Q).

The results of this study showed that the genotypic frequencies of the D151A polymorphism have no significant differences in the patient group in comparison with the control group (Table 2). Genotype frequencies of the R453Q polymorphism showed a significant difference between our groups. For R453Q polymorphism, the 'AG' genotype was associated with a 0.49-fold decreased risk of PCOS (P=0.023); Also, the A-allele compared to the G-allele reduced the risk of PCOS by approximately 0.56-fold (P=0.01, Table 2). We observed a 0.51-fold decrease in the risk of PCOS in the carriers of the genotypes 'AA' (for D151A), 'AA' (for R453Q), and also the carriers of the genotypes 'AA' (or D151A), 'AG' (for R453Q) (P=0.02). Also, there was no significant correlation among other combinations of the D151A and R453Q polymorphisms with PCOS risk (Table 3).

Table 2: Allele and genotype frequencies of *H6PD* D151A and R453Q

Genotypes\ Alleles	Controls n (%)	Patients n (%)	P value	OR (95% CI)
D151A				
AA	86 (86)	82 (82)		1
AC	13 (13)	16 (16)	0.53	1.29 (0.58-2.85)
CC	1 (1)	2 (2)	0.55	2.097 (0.186-23.57)
AC+CC	14 (14)	18(18)	0.44	1.35(0.63-2.89)
A	185 (92.5)	180 (90)		1
C	15 (7.5)	20 (10)	0.378	1.37 (0.68-2.76)
R453Q				
GG	45 (45)	63 (63)		1
AG	42 (42)	29 (29)	0.023	0.49 (0.27-0.91)
AA	13(13)	8 (8)	0.09	0.44 (0.17-1.15)
AA+AG	55 (55)	37 (37)	0.01	0.48 (0.27-0.85)
G	132 (66)	155 (77.5)		1
A	68 (34)	45 (22.5)	0.01	0.56 (0.36-0.88)

OR; Odds ratio, CI; Confidence interval, Pearson's chi-square (χ^2) test, (95% CI) with an unconditional logistic regression model.

Table 3: Calculation of the odds ratio between case and control groups for the combination of D151A and R453Q genotypes of *H6PD*

D151A	R453Q	OR (95% CI)	P value
AA	GG	1.63 (0.92-2.85)	0.089
AC+CC	GG	2.14 (0.77-5.94)	0.14
AA	AG+ AA	0.51 (0.28-0.9)	0.02*
AC+CC	AG+ AA	0.73 (0.24-2.19)	0.58

OR; Odds ratio, CI; Confidence Interval, Pearson's chi-square (χ^2) test, (95% CI) with an unconditional logistic regression model, and *; Statistically significant.

Discussion

Based on the importance of the single nucleotide polymorphisms in the development of the disease, such as PCOS and also, the important role of the *H6PD* gene in the human endocrine regulation (4), the *H6PD* (R453Q and D151A) variants were assessed in a population of Kurdish women from the West of Iran.

In the present study, we detected a significant difference in body mass index (BMI) between healthy controls and PCOS patients, although there were studies that reported a higher BMI and fat distribution could increase the PCOS risk (18-21).

Although the exact effect of H6PD on the PCOS phenotypes remains unknown, it seems that the H6PD has a specific role in influencing the function of the hypothalamic-pituitary-gonadal axis in humans (4). Some genetic studies reported that the *H6PD* variants are related to insulin resistance, obesity, and hyperandrogenism in PCOS patients (8, 22). However, the ovaries are the primary source of increased androgens in the syndrome, 20-30% of PCOS patients have adrenal androgen excess (23). In an animal study, the inactivation of the *H6PD* gene was associated with abnormalities of the hypothalamic-pituitary-adrenal axis, glucose homeostasis, and lipid metabolism (24). For the first time, in a population of Iranian women, we confirmed an association between the PCOS phenotype and the *H6PD* R453Q. In the present study, we observed that the 'A' allele of R453Q is less frequent in the patient group in comparison with the control group. Studies in different populations and ethnicities suggested that the H6PD R453Q polymorphism may be related the PCOS (4, 10, 14, 15). San Millan et al. (15) showed homozygosity for the 'G' allele of H6PD R453Q was more frequent in the patients with PCOS phenotype. In addition, they proposed that the variants of *H6PD* R453Q probably affect the adrenal activity involved in PCOS. Ju et al. (4) showed that the R453Q variant of the *H6PD* gene may affect the stability of the mRNA structure or its interaction with other macromolecules. *In vitro* study with H6PD as a bifunctional enzyme demonstrated that the R453Q preserved the two activities of this enzyme (25). However, *in vivo* consequences of this enzyme activity is not clear; thus the possibility can exist that the correlation of PCOS and R453Q may be due to variation in the activity of the H6PD enzyme or linkage imbalance with unknown variants of the *H6PD* gene (10). Despite several studies that confirm our results, several studies showed contradictory results. In the Ju et al. (4) study, the association between clinical features of PCOS and *H6PD* polymorphisms was investigated, and concluded that the FSH level and phenotype of hyperandrogenism of PCOS were associated with the 'AG' genotype of *H6PD* R453Q (rs6688832) in the PCOS patients. They also declared the 'GG' genotype and 'G' allele of R453Q likely have a protective role against PCOS. In another study, it was reported that the *H6PD* R453Q variant is associated with PCOS which has a modifying role in the phenotype of PCOS by adrenal hyperactivity improvement in the carriers of the 'A' allele (15).

A second genetic polymorphism in our study was *H6PD* D151A, which was not associated with the PCOS phenotype. Martínez-García et al. (10) reported that allele A151 was more frequent in obese women, particularly those with PCOS phenotype. So far, no functional studies reported the effects of D151A polymorphism on the activity of H6PD.

In addition, in our study, the interaction of the 'AA' genotype of D151A with the 'AA+ AG' genotypes of R453Q

significantly showed a reduction in reducing the PCOS phenotype development. It seems the D151 and R453 alleles, simultaneously, have a protective role against PCOS. Although, the interpretation of genetic studies should be done cautiously, and to confirm these results, studies in larger populations are necessary. San Millan et al. (15) declared that the compensatory hyperinsulinemia could elucidate the increased concentrations of androstenedione in the 'A' allele carriers (*H6PD*, D151A), whiles an increase in the local cortisol levels in the 'Q' allele carriers (*H6PD*, R453Q) would reduce the adrenal stimulation. This reduction in adrenal stimulation occurs by the protective effects of adrenocorticotropin against adrenal androgen excess and PCOS. The main limitation of the study lies in the fact that the BMI in the patient group was higher than in the control group. Although, the frequency of alleles between BMI groups was not significant (data were not shown).

Conclusion

The findings of this study suggest a protective effect of the R453 allele against the occurrence of PCOS. Due to the complexity of interaction in many genetic and environmental factors involved in this disorder, further studies are required to confirm these results in different ethnicity and larger sample size.

Acknowledgements

We thankfully acknowledge the Research Council of Kermanshah University of Medical Sciences for the financial support of this project. We are grateful to the participants for their willing participation and cooperation with us. This study was funded by the Kermanshah University of Medical Sciences, Kermanshah, Iran (Grant No. 97335). All the authors declare no conflict of interest.

Authors' Contributions

R.N., C.J.; Participated in conception, study design, and overall supervision. M.S., A.A.; Conducted molecular experiments (DNA extraction, PCR-RFLP analysis) and drafting. Y.A., M.C.F., E.B.; Contributed extensively to the statistical analysis, interpretation of the data, and the conclusion. All authors performed editing and approved the final version of this manuscript for submission, also participated in the finalization of the manuscript.

References

1. Naseri R, Barzingarosi E, Sohrabi M, Alimoradi Y, Cheraghian Fard M, Jalili C. The effect of leptin receptor gene polymorphisms (R223Q and P1019P) in susceptibility to polycystic ovarian syndrome in Kurdish women. Int J Fertil Steril. 2021; 15(2): 123-127.
2. Legro RS. Polycystic ovary syndrome. Phenotype to genotype. Endocrinol Metab Clin North Am. 1999; 28(2): 379-396.
3. Dumesic DA, Abbott DH, Padmanabhan V. Polycystic ovary syndrome and its developmental origins. Rev Endocr Metab Disord. 2007; 8(2): 127-141.
4. Ju R, Wu W, Tang Q, Wu D, Xia Y, Wu J, et al. Association analysis between the polymorphisms of HSD11B1 and H6PD and risk of polycystic ovary syndrome in Chinese population. PLoS One. 2015; 10(10): e0140326.
5. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. Endocr Rev. 2015; 36(5): 487-525.
6. Ehrmann DA, Liljenquist DR, Kasza K, Azziz R, Legro RS, Ghazzi MN. Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2006; 91(1): 48-53.
7. Padmanabhan V, Veiga-Lopez A. Animal models of the polycystic ovary syndrome phenotype. Steroids. 2013; 78(8): 734-740.
8. Qin K, Rosenfield RL. Mutations of the hexose-6-phosphate dehydrogenase gene rarely cause hyperandrogenemic polycystic ovary syndrome. Steroids. 2011; 76(1-2): 135-139.
9. Draper N, Walker EA, Bujalska IJ, Tomlinson JW, Chalder SM, Arlt W, et al. Mutations in the genes encoding 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. Nat Genet. 2003; 34(4): 434-439.
10. Martínez-García MA, San-Millán JL, Escobar-Morreale HF. The R453Q and D151A polymorphisms of hexose-6-phosphate dehydrogenase gene (H6PD) influence the polycystic ovary syndrome (PCOS) and obesity. Gene. 2012; 497(1): 38-44.
11. Krczal D, Ritter H, Kömpf J. Polymorphism of glucose dehydrogenase (GDH, EC 1.1.1.47): formal and population genetic data. Hum Genet. 1993; 91(3): 290-292.
12. Mason PJ, Stevens D, Diez A, Knight SW, Scopes DA, Vulliamy TJ. Human hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase) encoded at 1p36: coding sequence and expression. Blood Cells Mol Dis. 1999; 25(1): 30-37.
13. Hewitt KN, Walker EA, Stewart PM. Minireview: hexose-6-phosphate dehydrogenase and redox control of 11{beta}-hydroxysteroid dehydrogenase type 1 activity. Endocrinology. 2005; 146(6): 2539-2543.
14. Valkenburg O, Uitterlinden AG, Themmen AP, de Jong FH, Hofman A, Fauser BC, et al. Genetic polymorphisms of the glucocorticoid receptor may affect the phenotype of women with anovulatory polycystic ovary syndrome. Hum Reprod. 2011; 26(10): 2902-2911.
15. San Millán JL, Botella-Carretero JI, Alvarez-Blasco F, Luque-Ramírez M, Sancho J, Moghetti P, et al. A study of the hexose-6-phosphate dehydrogenase gene R453Q and 11beta-hydroxysteroid dehydrogenase type 1 gene 83557insA polymorphisms in the polycystic ovary syndrome. J Clin Endocrinol Metab. 2005; 90(7): 4157-4162.
16. Gadalla MA, Norman RJ, Tay CT, Hiam DS, Melder A, Pundir J, et al. Medical and surgical treatment of reproductive outcomes in polycystic ovary syndrome: an overview of systematic reviews. Int J Fertil Steril. 2020; 13(4): 257-270.
17. Moradi MT, Yari K, Khodarahmi R. A novel, efficient, fast and inexpensive DNA extraction protocol from whole blood applicable for studying drug-DNA interaction. J Rep Pharm Sci. 2014; 3(1): 80-84.
18. Pasquali R, Gambineri A, Biscotti D, Vicennati V, Gagliardi L, Colitta D, et al. Effect of long-term treatment with metformin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the polycystic ovary syndrome. J Clin Endocrinol Metab. 2000; 85(8): 2767-2774.
19. Good C, Tulchinsky M, Mauger D, Demers LM, Legro RS. Bone mineral density and body composition in lean women with polycystic ovary syndrome. Fertil Steril. 1999; 72(1): 21-25.
20. Mannerås-Holm L, Leonhardt H, Kullberg J, Jennische E, Odén A, Holm G, et al. Adipose tissue has aberrant morphology and function in PCOS: enlarged adipocytes and low serum adiponectin, but not circulating sex steroids, are strongly associated with insulin resistance. J Clin Endocrinol Metab. 2011; 96(2): E304-311.
21. Barber TM, Golding SJ, Alvey C, Wass JA, Karpe F, Franks S, et al. Global adiposity rather than abnormal regional fat distribution characterizes women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2008; 93(3): 999-1004.
22. Li Y, Chen C, Ma Y, Xiao J, Luo G, Li Y, et al. Multi-system reproductive metabolic disorder: significance for the pathogenesis and therapy of polycystic ovary syndrome (PCOS). Life Sci. 2019; 228: 167-175.
23. Yildiz BO, Azziz R. The adrenal and polycystic ovary syndrome. Rev Endocr Metab Disord. 2007; 8(4): 331-342.
24. Rogoff D, Ryder JW, Black K, Yan Z, Burgess SC, McMillan DR, et al. Abnormalities of glucose homeostasis and the hypothalamic-pituitary-adrenal axis in mice lacking hexose-6-phosphate dehydrogenase. Endocrinology. 2007; 148(10): 5072-5080.
25. Zhang YL, Zhong X, Gjoka Z, Li Y, Stochaj W, Stahl M, et al. H6PDH interacts directly with 11beta-HSD1: implications for determining the directionality of gluc ocorticoid catalysis. Arch Biochem Biophys. 2009; 483(1): 45-54.