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# Population-genetic Aspects of Breast Cancers and Association with Rh Factor in Selected Sample

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## ABSTRACT

**Background:** Breast cancer in women is the second most common and accounts for approximately 18% of all malignant tumors in women worldwide. The etiology of breast cancer is not clear enough. Starting from the assumption that the manifestation of breast cancer may have a multifactorial model, this article compares the population-genetic structure of patients (experimental group) with the population-genetic structure of healthy population (control group). **Objective:** The aim of the study was to examine the possible genetic basis of the Rh factor relationship with selected homozygous-recessive traits of females with breast cancer, and to diagnose the probability (assess the risk) of developing the disease in healthy women by analyzing homozygous-recessive traits (HRT). **Methods:** This are an anthroposcopic-qualitative study that included two groups of subjects, experimental and control (a total of 80 subjects). An analysis of the percentages within each group was performed using the Chi-square test. The results are presented in tables, and the accepted level of significance is at the level of  $p < 0.05$ . **Results:** In the group of Rh+ subjects, the correlation of this type of Rh factor with the breast cancer was proven, given the frequency of the phenotype of homozygous-recessive traits in them. A statistically significant difference was found for 4 traits, and three are also close to the set significance level. In subjects with Rh- factor, a statistically significant difference was found for only one trait (absence of mallets on the phalanges). **Conclusion:** Although the number of subjects was relatively small, we can conclude that in the experimental group a higher frequency of recessive phenotypes for the examined traits was recorded, which indicates the genetic load of the subjects from this group. Correlation with Rh factor was observed in the case of subjects of the experimental group with Rh+ factor.

**Keywords:** breast cancer, recessive trait, homozygous recessive, Rh factors.

## 1. BACKGROUND

Breast cancer (BC) is the most commonly diagnosed cancer in women worldwide with more than 2 million new cases in 2020 (1). Its incidence and death rate have increased in the last three decades due to changing risk profile profiles, better cancer registration and detection. The number of BC risk factors is significant and includes both modified and non-modified factors. Currently, about 80% of patients with BC are individuals aged over 50 years. (2).

Breast cancer is the most common malignant tumor in women in the world as a whole, which occurs when normal breast glandular cells change their properties and begin to grow uncontrollably, multiply and destroy the surrounding healthy tissue (3).

Breast cancer in women is the second most common type of cancer and accounts for about 18% of all malignant tumors in women worldwide (4). In developed countries (including BiH) it is even at the first place.

There are numerous classifications of malignant breast tumors (5, 6). The genes responsible for hereditary breast cancer (discovered in 1994 and 1995) are called Breast Cancer 1 and Breast Cancer 2, respectively, BRCA-1 and BRCA-2 (7). Women who inherit these mutations are at high risk of getting sick from this disease, according to some experts up to 80%. BRCA-1 mutations also increase the risk of ovarian cancer (8). These are large genes and their mutations are very numerous and affect different sites in the DNA chain (9, 10).

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TRAIT	EXPERIMENTAL GROUP						CONTROL GROUP					
	N (40)	Rh+ (34)	%	Rh- (6)	%	p	N (40)	Rh+ (33)	%	Rh- (7)	%	P
Flat scalp	25	21	61.76	4	66.66	0.600	17	16	48.48	1	14.28	0.105
Two flowers in the hair	7	7	20.58	0	0	0.289	0	0	0	0	0	0
Soft hair	34	31	91.17	3	50.00	0.033	25	20	60.60	5	71.42	0.467
Real hair	27	24	70.58	3	50.00	0.293	22	17	51.51	5	71.42	0.297
Tied earlobe	16	14	41.17	2	33.33	0.544	24	18	54.54	6	85.71	0.134
An ear without Darwin's nodule	24	19	55.88	5	83.33	0.212	21	18	54.54	3	42.85	0.441
Thin lips	27	22	64.70	5	83.33	0.351	26	23	69.69	3	42.85	0.179
Retracted teeth	7	6	17.64	1	16.66	0.721	3	3	9.09	0	0	0.552
Retracted chin	4	4	11.76	0	0	0.507	3	2	6.06	1	14.28	0.448
Absence of mallets	24	21	61.76	3	50	0.456	24	20	60.60	4	57.14	0.592
Narrow nostrils	23	20	58.82	3	50	0.551	22	19	57.57	3	42.85	0.383
Inability to bend the tongue longitudinally	16	12	35.29	4	66.67	0.160	13	10	30.30	3	42.85	0.408
Inability to bend the tongue backwards	11	11	32.35	0	0	0.128	4	3	9.09	1	14.28	0.552
Speech defect "R"	8	7	20.59	1	16.67	0.656	1	1	3.03	0	0	0.825
Right thumb over left	20	17	50	3	50	0.669	23	18	54.54	5	71.42	0.351
Mobility of the distal part of the thumb	8	7	20.58	1	16.66	0.656	6	6	18.18	0	0	0.289
Ability to reach the forearm with the thumb	3	2	5.88	1	16.66	0.394	9	7	21.21	2	28.57	0.504
Way of crossing arms, R-phenotype	19	17	50	2	33.33	0.381	22	19	57.57	3	42.85	0.383
Absence of mallets on the phalanges	26	23	67.64	3	50	0.346	34	27	81.81	7	100	0.289
Three tendons at the root of the fist	32	27	79.41	5	83.33	0.656	28	22	66.66	6	85.71	0.306

**Table 1. Frequency of HRT in the examined groups in relation to Rh factor**

Breast cancer is an important public health problem. Epidemiological studies have identified several risk factors for breast cancer, such as age, heredity, diet and lifestyle, reproductive activity, socioeconomic status and racial affiliation (11). Experimental molecular genetic studies have shown the role of mutagenic factors, sex hormones, free radicals and oncogenes.

In order to examine the role of genetic factors in the development of breast cancer, numerous population-genetic and cytogenetic studies, studies of twins, and the association studies of this disease with certain blood-group systems and antigens have been conducted (12). Although there have been tremendous advances in elucidating genetic risk factors underlying both familial and sporadic breast cancer, much of the genetic contribution to breast cancer etiology remains unknown (13). The genetics of breast cancer risk in the post-genome era: thoughts on study design to move past BRCA and towards clinical relevance (14).

Starting from the assumption that the manifestation of breast cancer can be based on the genetic dispositions of certain individuals, it seemed interesting to compare the population-genetic structure of cancer patients with the population-genetic structure of healthy individuals (15-18).

Assumptions that the manifestation of breast cancer may be based on genetic predispositions, made it interesting to compare the population-genetic structure

of breast cancer patients with the population-genetic structure of healthy individuals.

A very suitable manner to establish possible differences between these two groups could be to examine the most striking morphological and physiological characteristics of humans, which could serve as parameters of population-genetic structure. The assumption that at the level of the examined phenotypes differences could be expected between patients with breast cancer and healthy (control) samples and can be explained in two ways:

Genes on which the manifestation of breast cancer or resistance to the factors that cause this disease can simultaneously have a pleiotropic effect on a number of other characteristics and thus manifest themselves comparatively through them;

Genetic loads resulting from increased recessive homozygosity for a number of characteristics, in persons with breast cancer are the cause of reduced resistance to external factors that cause this disease.

## 2. OBJECTIVE

\* Examine the possible genetic basis of the Rh factor relationship with selected homozygous-recessive traits of females with breast cancer, and pre-diagnose the probability (assess the risk) of developing the disease in healthy women by analyzing homozygous-recessive traits (HRT).

TRAIT	EXPERIMENTAL GROUP (34)			CONTROL GRUPO (33)			P
	Present	Absent	%	Present	Absent	%	
Flat scalp	21	13	61.67	16	17	48.48	0.274
Two flowers in the hair	7	27	20.59	0	33	0	0.006
Soft hair	31	3	91.18	20	13	60.61	0.003
Real hair	24	10	70.59	17	16	51.52	0.109
Tied earlobe	15	19	44.12	18	15	54.55	0.393
An ear without Darwin's nodule	19	15	55.88	18	15	54.55	0.912
Thin lips	22	12	64.71	23	10	69.70	0.664
Retracted teeth	6	28	17.65	3	30	9.09	0.253
Retracted chin	4	30	11.76	2	31	6.06	0.351
Absence of mallets	21	13	61.76	20	13	60.61	0.922
Narrow nostrils	20	14	58.82	19	14	57.58	0.918
Inability to bend the tongue longitudinally	12	22	35.29	10	23	30.30	0.664
Inability to bend the tongue backwards	11	23	32.35	3	30	9.091	0.019
Speech defect "R"	7	27	20.59	1	32	3.03	0.027
Right thumb over left	17	17	50.00	18	15	54.55	0.710
Mobility of the distal part of the thumb	7	27	20.59	6	27	18.18	0.803
Ability to reach the forearm with the thumb	2	32	5.88	7	26	21.21	0.068
Way of crossing arms, R-phenotype	17	17	50.00	19	14	57.58	0.534
Absence of mallets on the phalanges	23	11	67.65	27	6	81.82	0.183
Three tendons at the root of the fist	27	7	79.41	22	11	66.67	0.239

**Table 2. Incidence of HRT in Rh+ subjects of the control and experimental groups**

TRAIT	EXPERIMENTAL GROUP			CONTROL GROUP			p
	Present	Absent	%	Present	Absent	%	
Flat scalp	4	2	66.67	1	6	14.29	0.083
Two flowers in the hair	0	6	0	0	7	0	0
Soft hair	3	3	50.00	5	2	71.43	0.413
Real hair	3	3	50.00	5	2	71.43	0.413
Tied earlobe	2	4	33.33	6	1	85.71	0.86
An ear without Darwin's nodule	5	1	83.33	3	4	42.86	0.179
Thin lips	5	1	83.33	3	4	42.86	0.179
Retracted teeth	1	5	16.67	0	7	0	0.462
Retracted chin	0	6	0	1	6	14.29	0.538
Absence of mallets	3	3	50.00	4	3	57.14	0.617
Narrow nostrils	3	3	50.00	3	4	42.86	0.617
Inability to bend the tongue longitudinally	4	2	66.67	3	4	42.86	0.383
Inability to bend the tongue backwards	0	6	0	1	6	14.29	0.538
Speech defect "R"	1	5	16.67	0	7	0	0.261
Right thumb over left	3	3	50.00	5	2	71.43	0.413
Mobility of the distal part of the thumb	1	5	16.67	0	7	0	0.462
Ability to reach the forearm with the thumb	1	5	16.67	2	5	28.57	0.563
Way of crossing arms, R-phenotype	2	4	33.33	3	4	42.86	0.587
Absence of mallets on the phalanges	3	3	50.00	0	7	0	0.033
Three tendons at the root of the fist	5	1	83.33	6	1	85.71	0.731

**Table 3. Incidence of HRT in Rh-subjects of the experimental and control groups**

\* Determine the existence of a potential statistically significant relationship between one or more HRTs with a certain type of Rh factor in patients.

\* Statistically process the data, determine the frequency of occurrence of certain HRTs and their relationship with Rh factor in subjects with breast cancer in relation to the control group.

\* Determining the existence of a potential statistically significant relationship between one or more HRTs with a particular type of Rh factor in obese individuals.

### 3. MATERIAL AND METHODS

This anthroposcopic-qualitative study includes two groups of subjects.

**Experimental group:** Its included 40 female subjects with breast cancer. The criteria for inclusion in the experimental group was confirmation of the diagnosis based on official medical documentation, PH finding. The experimental group did not include persons who had other diagnoses related to malignant processes.

**Control group:** Its included 40 healthy female subjects. The control group was obtained by random sampling, taking into account that there is no familial predisposition for the development of breast cancer.

All respondents signed an informed consent form for participation in the study, which applied methodology that ensures their anonymity. A morphophysiological approach was performed to collect data. A variant of the phenotype of the studied traits was determined for each subject by direct observation and interviewing.

#### Statistical analysis

Statistical evaluation of the study results yielded more complete and comprehensive results that showed whether there was a significant statistical correlation between recessive alleles of selected HRT and Rh factor type in patients with breast cancer.

## 4. RESULTS

The “HRT test” was used in this study, which includes qualitative morphological and functional characteristics that are under the control of genetic factors and cover all regions of the human body: hair characteristics, facial features, pronunciation, tongue mobility, finger characteristics, hand characteristics and finger mobility (19-22).

Table 1 present the frequency of HRT in the experimental and control groups in relation to Rh factor. The results are presented in absolute number, percentage and the p value.

Table 2 shows the frequency of HRT in the experimental and control groups in relation to the Rh+ factor of the subjects. The values are expressed in absolute numbers, percentage representation, and the p value.

Table 3 shows the frequency of HRT in the experimental and control groups in relation to the Rh factor of the subjects. The values are expressed in absolute numbers, percentage representation, and the p value.

## 5. DISCUSSION

The etiology of breast cancer has not yet been fully elucidated. The efforts of scientists to reveal the mechanism of malignancy and risk factors, and certain markers are very widespread.

Molecular techniques are the focus of this research. However, genetic predisposition or risk of diseases are also “marked” through the phenotypic manifestation of genes (21, 22).

Population genetic studies of traits through the HRO test during our study are not processed in this way in the world, so the data obtained in our study can be considered a new understanding of the etiology of breast cancer.

Thus, great attention is paid to HRT, not only in breast cancer, but also in others diseases (23). Possibility of correlation of several genes (which affects the phenotypic expression of one trait). These genes may be involved in regulatory processes of resistance to the studied disorder (24).

The obtained results show an increased genetic load through an increase in recessive homozygosity, which in

patients may be the cause of reduced resistance to external factors (25).

These types of research present similar results for some other diseases, such as tumors of the urinary tract, tumors of other locations, endemic nephropathy, spinal dysraphism (26).

The age difference in the examined groups does not affect the obtained results since the analyzed traits do not change over a lifetime.

The correlation between HRT and Rh factors showed that a larger number of patients with Rh+ factor, who had significant statistical differences in the examined HRTs (found a difference in 4 examined HRTs).

The difference in the population-genetic structure of the experimental and control group with Rh-factor is in only one feature. Therefore, we can state that there is no significant Rh factor in the examined sample associations with HRT in breast cancer patients.

The most likely mechanism in the progress of an association among “ABO” and “Rhesus” blood types and the incidence of breast cancer has not been established yet. The current genome-wide association studies suggest that the “ABO” blood type antigen increases the incidence of breast cancer. The genetic factors are most likely involved in the etiology of breast cancer (27-30).

## 6. CONCLUSIONS

Based on all the above, the following can be concluded:

\* The result in the group of Rh+ subjects indicate the correlation of this type of Rh factor with the development of breast cancer, given the frequency of the phenotype of homozygous-recessive traits in them.

\* A statistically significant difference was found for 4 traits, and three are still close to the significance limit. Females with Rh+ factor are at higher risk of developing breast cancer.

\* In subjects with Rh- factor, a statistically significant difference was found for only one trait (absence of hair on the phalanges), so that the correlation of this Rh factor with breast cancer has not been established.

\* Although the number of respondents was relatively small, the conclusion is that it is in the experimental group a higher frequency of recessive phenotypes of the examined traits was noted, indicating genetic load of respondents from this group. Correlation with Rh factor was observed in the case of the subjects experimental groups with Rh+ factor.

\* It is necessary to expand the research, both in terms of the number of respondents and the introduction of new methodologies.

\* The application of the HRO test in the future may be useful in detecting genotypes susceptible to various diseases.

- **Patient Consent Form:** All participants gave informed consent prior participation.
- **Author's Contribution:** P.T. and K.G. contributed to the conception and design of the study. P.T. performed data acquisition and statistical analysis. P.T. and K.G. were involved in

article drafting. N.A. contributed in manuscript revision and interpretation of data. P.A. contributed in samples collection. All authors have approved the final version of the manuscript.

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## REFERECES

1. Łukasiewicz S, Czezelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast Cancer-Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies - An Updated Review. *Cancers*. 2021; 13(17): 4287.
2. Benz CC. Impact of aging on the biology of breast cancer. *Crit Rev Oncol*. 2008; 66: 65-74.
3. Boranić M. Etiologija i patogeneza tumora dojke. *Med Vjesn*. 2006; 38(1-4): 33-42.
4. Mavaddat N, Pharoah PD, Michailidou K, Tyrer J, Brook MN, Bolla MK, Wang Q, Dennis J, Dunning AM, Shah M, et al.; Prediction of breast cancer risk based on profiling with common genetic variants. *J Natl Canc Inst*. 2015; 107(5).
5. Jelenić J. Određivanje fenotipa raka dojke imunohistokemijskim bojenjem, završni rad. Sveučilište Josipa Jurja Strossmayera u Osijeku, Medicinski fakultet Osijek, 2017.
6. Perou CM, Sorlie T, Eisen MB, Van de Rijn M, Jeffrey SS et al. Molecular portraits of human breast tumours. *Nature*. 2000; 406: 747-752.
7. McGuire A, Brown JAL, Malone C, McLaughlin R, Kerin MJ. Effects of Age on the Detection and Management of Breast Cancer. *Cancers*. 2015; 7: 908-929.
8. Lee S, Abecasis GR, Boehnke M, Lin X. Rare-variant association analysis: study designs and statistical tests. *Am J Hum Genet*. 2014; 95(1): 5-23.
9. Eminović I. Biologija kancera; autorizovana skripta, PMF UNSA, 2019.
10. Kuchenbaecker KB, Hopper JL, Barnes DR, et al. Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. *JAMA*. 2017; 317(23): 2402-2416.
11. Momenimovahed Z, Salehiniya H. Epidemiological characteristics of and risk factors for breast cancer in the world. *Breast Cancer*. 2018; 10(11): 151-164.
12. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, *CA Cancer J Clin*. 2014; 64: 9-29.
13. Skol AD, Sasaki MM, Onel K. The genetics of breast cancer risk in the post-genome era: thoughts on study design to move past BRCA and towards clinical relevance. *Breast Cancer Res*. 2016; 18: 99.
14. Shiovitz S, Korde LA. Genetics of breast cancer: A topic in evolution. *Ann. Oncol*. 2015; 26: 1291-1299.
15. Siddiq A, Couch FJ, Chen GK, Lindstrom S, Eccles D, Millikan RC, Michailidou, K, Stram DO, Beckmann L, Rhie SK, et al. A meta-analysis of genome-wide association studies of breast cancer identifies two novel susceptibility loci at 6q14 and 20q11. *Hum Mol Genet*. 2012; 21(24): 5373-5384.
16. Garcia-Closas M, Couch FJ, Lindstrom S, Michailidou K, Schmidt MK, Brook MN, Orr N, Rhie SK, Riboli E, Feigelson HS, et al. Genome-wide association studies identify four ER negative-specific breast cancer risk loci. *Nat Genet*. 2013; 45(4): 392-398.
17. Vachon CM, Pankratz VS, Scott CG, Haeberle L, Ziv E, Jensen MR, Brandt K,
18. Garaj-Vrhovac V. Analysis of mutations in somatic cells Arh Hig Rada Toksikol, 51Supplement: 2000; 115-124.
19. Hadžiselimović R., Pojskić N. Uvod u humanu imunogenetiku. Institut za genetičko inženjerstvo i biotehnologiju (IN-GEB), Sarajevo, 2005.
20. Hadžiselimović R. Bioantropologija – Biodiverzitet recentnog čovjeka. Institut za genetičko inženjerstvo i biotehnologiju (IN-GEB), Sarajevo, 2005.
21. Ahmić A, Tursunović A, Bašić A, Hamidović H, Hadžihalilović J, Pojskić N, Hadžiselimović R. Genetička struktura populacija domicilnog i migratornog stanovništva sa područja tuzlanske regije s obzirom na neka statičko i dinamičko morfološka svojstva. *Glasnik Antropološkog društva Srbije*. 2012; 47: 173-180.
22. Nikolić T. Zastupljenost HRO osobina kod stanovnika Svrjljiga i Niša. Univerzitet u Nišu, Prirodno-matematički fakultet, Departman za biologiju. Master rad, Niš, 2017.
23. Pesut D. Susceptibility to lung tuberculosis – a population-genetic study. *Medicinski Pregled*. 2004; 57: 21-24.
24. Cvjeticanin S, Marinković, Genetic variability and frequencies of ABO blood types among different samples of patients from Serbia. *Korean Journal of Genetics*. 2005; 27(1): 35-40.
25. Cvjeticanin S, Nikolić D, Petronić I., Jekić B, Damjanović T, Ćirović D, Radlović V, Knežević T. Utvrđivanje stepena homozigotnosti kod bolesnika sa spinalnim disrafizmima. *Srpski arhiv za celokupno lekarstvo*. 2008; 136: 519-523.
26. Čukuranović R, Marinković D, Pavlović S, Vasović Lj, Vlajković S, Daković-Bjelaković M, Stojanović V, Jovanović I., Ugrenović S, Zdravković D. Populaciono – genetički aspekti Balkanske endemske nefropatije u slivu Južne Morave. *Acta Medica Mediana*. 2004; 43: 11-18.
27. Garratty G. Blood groups and disease: a historical perspective. *Transfus Med Rev*. 2000; 14: 291-301.
28. Anstee DJ. The relationship between blood groups and disease. *Blood*. 2010; 115: 4635-4643.
29. Meo SA, Suraya F, Jamil B, et al. Association of ABO and Rh blood groups with breast cancer. *Saudi J Biol Sci*. 2017; 24(7): 1609-1613.
30. Nayak SK. ABO blood groups in different diseases. *J Ind Med*, 1997; 87: 449-452.