2024. 1
ISSN 3007-0090

Journal of General Practice Research and Translation

ISSN 3007-0090
9 773007 009000 >

Evaluation of Genetics of Obesity and MC4R Deficiency: A Gene-oriented Approach to Obesity

Onur Oral1* George Nomikos[2] Nikitas Nomikos[3]

1.Ege University Faculty of Sports Sciences, Department of Health Sciences and Sports, Izmir, Turkey

2.Chios Hospital, Department of Orthopaedic Surgery, Chios, Greece

3.Medical School & School of Physical Education and Sport Science, National & Kapodistrian University of Athens, Greece

ABSTRACT

Obesity is a multifactorial and complex health problem that is affected by several factors including genetic, environmental, social, behavioural, and biological aspects.Even though the influence of other environmental and behavioural factors such as sedentary lifestyle, high-calorie nutritional intake, and the inadequate expenditure of energy are acknowledged as important aspects that cause obesity, the issue of inheritance is indisputable. The study aims to investigate the effects of inheritance on obesity and examine how understanding and detecting genetic reasons behind obesity may benefit the treatment and prevention of the obesity epidemic. The relationship between common gene variants and obesity is now being studied through the emergence of GWAS. It is undeniable that genetic mutations and gene deficiencies particularly MC4R deficiency are significant factors.The process of detecting genes that create a tendency to obesity is currently being studied. It may be possible to prevent and treat obesity with the detection of certain genes.

Keywords: Obesity;Genetics of obesity;MC4R;Mutation

1.Introduction

Obesity is a public health concern worldwide. Obesity cases show a significant increase in both developed and developing countries. The disease is now considered an epidemic since it threatens public health in a global sense.Not only obesity influence societies in terms of health,but also social and economic terms. Obesity is known to be a factor that induces several other diseases such as stroke, hypertension,reflux,several cancer types,liver cirrhosis T2D, depression, etc. Therefore, it is argued that obesity decreases average lifetime . Exercise has many beneficial effects, leads to less telomere attrition and may diminish the risk of cancer, these two outcomes are possible resulted by a reduction in oxidative stress and chronic inflammation. Hippocrates was the first to recognize the need for a balanced diet and exercise and the fact that different age has different needs. Hippocrates' innovative spirit laid the foundations of modern medicine and the wellbeing movement: Exercise Is Medicine (EIM) ®,which has been inspired-adopted by scientific institutions such as the American College of Sports Medicine (ACSM), the

American Medical Association (AMA) and Harvard Medical School.

Obesity is a multifactorial and complex health problem. Even though a wide number of studies indicate that genetics played a role for 40 to 70% of obese patients by provoking weight gain, it cannot be suggested that genetics only cause this disease. Obesity is affected by several factors including genetic, environmental, social, behavioural, and biological aspects. In this sense, it is important to emphasize that one factor only does not directly lead to obesity. For instance, it is possible for an individual who has a genetic tendency to obesity, to stay healthy and have a normal BMI by adjusting their physical and nutritional habits. In fact, in a study, Xiang et al. compared those who carry a genetic variant that may cause obesity and the control group without the variant. The study indicated that following the right nutrition program and with adequate physical activity, a person with the genetic variant can reach a normal weight and live a healthy life. For this reason, while it is severely important to examine the impact of inheritance on obesity, it is also important to understand the interaction of the genes with the environment and other aspects.

1.1Types of Obesity

In a general sense, obesity can be divided into categories based on its etiology. These categories include monogenic obesity, syndromic obesity, and polygenic/common obesity. Monogenic obesity, which is also known as single gene obesity, is not a common but quite severe type of obesity. The reason for monogenic obesity is a single gene mutation or deficiency. Syndromic obesity is considered to be linked to mental retardation and certain abnormities in the development of organs. Polygenic obesity is caused by multiple polymorphic genes. It is a common type of obesity that is known to be seen more frequently in society and increases the risk for other diseases.

1.2Genetic Basis of Obesity

The importance of inheritance in the practice of medicine and community health has gained quite significance. Since inheritance is considered an issue significant to community health, genetics pose a crucial factor for urgent health problems that threaten people's health. Obesity, being of those public health problems, is considered a severe risk factor for other diseases such as coronary heart disease (CHD).

Table 1. Single genes are known to be linked to obesity

NAME	GENE	MIM	MODE of INHERITANCE	CHROMOSOMAL POSITION
Leptin	LEP	164160	AR	7q32.1
Leptin receptor	LEPR	601007	AR	1p31.2
Proopiomelanocortin	POMC	176830	AR	2p23.2
Melanocortin 4 receptor	MC4R	155541	AD/AR	18q21.32
Single-minded Drosophila Homologue-1	SIM1	603128	AD	6q16.3
Nurotrophic Tyrosine Kinase Receptor Type 2	NTRK2	600456	AD	9q21.33
Kinase suppressor of Ras2	KSR2	610737	AD	12q24.22-q24.23
Carboxypeptidase	CPE	114855	AD	4q32.3
Proconvertase 1	PCSK1	162150	AR	5q15
Brain Derived Neurotropic factor	BDNF	113505	AD	11p14.1
SH2B adaptor protein	SH2B1	608937	AD	16p11.2
Tubby, Homogue of Mouse	TUB	601197	AR	11p15.4

AD= Autosomal dominant, AR = Autosomal recessive.
For detailed information and references, refer to Online Mendelian Inheritance in Man using the MIM number: https://www.omim.org

The fact that obesity has a genetic origin is indisputable. Even though the influence of other environmental and behavioural factors such as sedentary lifestyle, high-calorie nutritional intake, and the inadequate expenditure of energy are acknowledged as important aspects that cause obesity, the issue of inheritance is a considerable factor.

Earliest findings of the association between obesity and inheritance date back to 2007 . Along with the improvements in technic and analysis methods, genome-wide association studies (GWAS) started. GWAS is an approach that helps scientists to reveal and discover the genetic reasons behind certain diseases. According to GWAS, common variants altogether can pass on through the family (International HapMap Consortium, 2005) . With this information, scientists were able to detect nearly 80% of common gene variations.

1.2.1MC4R Deficiency

The melanocortin-4-receptor (MC4R) is encoded by the MC4R gene. It is a G-protein coupled seven-transmem- brane receptor G protein-coupled receptor and is proven to be associated with obesity disease. It regulated

the nutritional behaviour in the hypothalamus . α-melano- cyte-stimulating hormone (α-MSH) binds and activates MC4R and this helps control appetite. Appetite regulation is linked to MC4R. Thus, one of the most common reasons for obesity based on genetics is certain mutations in MC4R. This type of obesity is quite prevalent in societies at a rate of 0.5-6%.

2.Discussion

Many researchers are studying genetics' influence on obesity and twin studies are quite common. These studies suggest that a similar phenotype is observed in the other twin or either of the parents, indicating the inheritability of obesity. According to several studies conducted on families, twins, and adopted family members, BMI is affected by genetics by 70-80%. Similar findings are seen in studies conducted on societies from various ethnic groups.

According to the studies in the field of epidemiology, as the degree of affinity lowers, so does the risk of obesity. Therefore, it is argued that inheritance is an important factor. In twin studies, it is indicated that dizygotic twins demonstrate a concordance rate by less than half of the monozygotic twins (~ 0.68 vs~0.28).

Similarly, in studies conducted on families with adopt- ed children, it was observed that the adopted children's BMI is more proximate to their biological parent compared to the BMI of their legal parents. This significantly emphasizes that environmental factors such as a mutual home environment are still important but the influence of inheritance on obesity is undeniable.

Farooqi et al. examined the MC4R deficiency or mutations and obesity relationship in a study conducted with families. Endocrine and metabolic analyses were performed on subjects. The study reported that 5.8% of the patients that suffered from obesity showed MC4R mutations. Farooqi et al. indicated in this wide-ranging study that the monogenic type of obesity is associated with MC4R deficiency. However, it is noted that in similar studies conducted on diverse ethnic populations, this rate was lower.

Bonnefond et al. reported that even though obese individuals that have monogenic MC4R mutations are suitable for bariatric operations. These patients lose less weight after the operation compared to people that have common obesity. Bonnefond et al. Lapsen et al. Collet et al. conducted a study on patients with MC4R mutations and a control group. While the group carrying the mutation was treated with Setmelanotide which is known to be an MC4R agonist, they lost an average weight of 3,5 kilograms. However, the control group receiving placebo treatment lost 0,85 kilograms within the same period.

Table 2. Comparison of phenotypic features of monogenic forms of obesity

Gene	Obesity	Birth weight	Endocrine abnormalities	Hyperphagia	Inheritance	Chromosome
LEP	Severe	Normal	Low leptin Hypogonadism High thyroid-stimulating hormone High insulin	+	Recessive	7q31.3
LEPR	Severe	?	High leptin Pituitary dysfunction Hypogonadotrophic hypogonadism Hypothalamic hypothyroidism Sympathetic dysfunction High insulin	+	Recessive	1p31
POMC	Severe	Norma	Red hair pigmentation ACTH deficiency,hypocortisolism Lowa-MSH	+	Recessive	2p23.3
PC1	Severe	?	Hypogonadotrophic hypogonadism Hypocortisolism High proinsulin, low insülin Postprandial hypoglycemia High POMC	?	Recessive	5q1.5–2.1
MC4-R	Severe	Normal	Not observed	+	Dominant	18q22
NROB2	Mild	High	Mild hyperinsulinemia	-	Dominant	1p36.1

3.Conclusions

Obesity is a multifactorial epidemic and it is the result of various factors. Genetics plays a crucial role in the occurrence of the disease. The relationship between common gene variants and obesity is now being studied through the emergence of GWAS. Regarding our extensive literature review, it is understood that it is undeniable that genetic mutations and gene deficiencies particularly MC4R deficiency are significant factors. The process of detecting genes that create the tendency to obesity is currently being studied. It may be possible to prevent and treat obesity with the detection of certain genes. Investigation of genetic factors on obesity should be further studied for future and current patients to maintain a healthier life.

References

[1]Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C.et al. (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet; 384: 766-781.

[2]Bluher, M. (2016). Adipose tissue inflammation: a cause or consequence of obesity-related insulin resistance?.Clin Sci (Lond).; 130: 1603-1614.

[3]Long, E., Beales, I.L. (2014). The role of obesity in oesophageal cancer development.Ther Adv Gastroenterol; 7: 247-268.

[4]Rosso, N., Chavez-Tapia, N.C., Tiribelli, C., Bellen- tani, S. (2014).Translational approaches: from fatty liver to non-alcoholic steatohepatitis.World J Gastroenterol; 20: 9038-9049.

[5]Nomikos NN, Nikolaidis PT, Sousa CV, Papalois AE, Rosemann T, Knechtle B. Exercise, Telomeres, and Cancer: “The Exercise-Telomere Hypothesis”. Front Physiol. 2018;9:1798. Published 2018 Dec 18. DOI:10.3389/fphys.2018.01798.

[6]Nomikos, N., Trompoukis, C., Lamprou, C., & No-mikos, G. (2016). The Role of Exercise in Hippocratic Medicine. American Journal of Sports Science and Medicine, 4(4), 115-119.

[7]Nomikos, N., Lamprou C. (2021). Proceedings of 47 Panhellenic Medical Congress. The reports about dietary and exercise in the texts of Hippocrates.

[8]Chung, W.K., Leibel, R.L. (2008). Considerations

regarding the genetics of obesity. Obesity (Silver Spring). Suppl 3:S33-9.

[9]Wood, A.C. (2018). Appetitive Traits: Genetic Contributions to Pediatric Eating Behaviors. In: Pediatric Food Preferences and Eating Behaviors. Elsevier; 127-146.

[10]Scuteri, A., Sanna, S., Chen, W.M., et al. (2007). Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. PLoS Genet, 3:e115.

[11]Xiang, L., Wu, H., Pan, A., et al. (2016). FTO genotype and weight loss in diet and lifestyle interventions: a systematic review and meta-analysis, 2. Am J Clin Nutr. ;103(4):1162-1170.

[12]Herrera B.M., Lindgren C.M. (2010). The Genetics of Obesity. Curr Diab Rep 10:498–505 DOI 10.1007/ s11892-010-0153-z.

[13]Thaker V. V. (2017). Genetic And Epigenetic Causes Of Obesıty. Adolescent medicine: state of the art reviews, 28(2), 379-405.

[14]Mangum, B.P., Mangum, T.L. (2018). Gene-environment interactions and the genetic epidemiology of obesity: correlates for preventative medicine. J Obes Metab Disord.;1(1):25-28.

[15]Torres, K. Y. (2010). Chronic Disease Epidemiology and Control, 3rd Edition. Preventing Chronic Dis- ease, 8(1), A25.

[16]Scuteri, A., Sanna, S., Chen, W.M., et al. (2007). Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. PLoS Genet, 3:e115.

[17]Frayling, T.M., Timpson, N.J., Weedon, M.N., et al. (2007). A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science, 316:889-894.

[18]International HapMap Consortium (2005). A haplo-type map of the human genome. Nature, 437:1299-1320.

[19]Barrett, J.C., Cardon, L.R. (2006). Evaluating cover- age of genome-wide association studies. Nat Genet, 38:659-662.

[20]Pe'er, I., de Bakker, P.I., Maller, J., et al. (2006). Evaluating and improving power in whole-genome association studies using fixed marker sets. Nat Genet, 38:663-667.

[21]LeRoith, D., Taylor, S.I., and Olefsky, J.M. (2004). Diabetes Mellitus: A Fundamental and Clinical Text (3rd Ed.) Lippincott Williams & Wilkins.

[22]Mountjoy, K.G., Wong, J. (1997). Obesity, diabetes and functions for proopiomelanocortin-derived pep- tides. Mol Cell Endocrinol.;128(1-2):171-7.

[23]Fan, W., Boston, B.A., Kesterson, R.A., Hruby, V.J., Cone, R.D. (1997). Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature. ;385(6612):165-8.

[24]Nowacka-Woszuk, J., Cieslak, J., Skowronska, B., et al. (2011). Missense mutations and polymorphisms of the MC4R gene in Polish obese children and adolescents in relation to the relative body mass index. J Appl Genet.;52(3):319-23.

[25]Wangensteen, T., Kolsgaard, M.L.P., Mattingsdal, M., et al. (2009). Mutations in the melanocortin 4 receptor (MC4R) gene in obese patients in Norway. Exp Clin Endocrinol Diabetes.;117(6):266-73.

[26]Stutzmann, F., Tan, K., Vatin, V., et al. (2008). Prevalence of melanocortin-4 receptor deficiency in Europeans and their age-dependent penetrance in multi-generational pedigrees. Diabetes.;57(9):2511-8.

[27]Elks, C.E., Den Hoed, M., Zhao, J.H., et al. (2012). Variability in the heritability of body mass index: a systematic review and meta-regression. Front Endocrinol.;3:29.

[28]Silventoinen, K., Jelenkovic, A., Sund, R., et al. (2017). Differences in genetic and environmental variation in adult BMI by sex, age, time period, and region: an individual-based pooled analysis of 40 twin cohorts. Am J Clin Nutr.;106(2):457-466.

[29]Llewellyn, C., Wardle, J. (2015). Behavioral susceptibility to obesity: gene– environment interplay in the development of weight. Physiol Behav.;152:494–501.

[30]Allison, D.B., Kaprio, J., Korkeila, M., Koskenvuo, M., Neale, M.C., Hayakawa, K. (1996).The heritability of body mass index among an international sample of monozygotic twins reared apart. Int J Obes Relat Metab Disord.; 20: 501-506.

[31]Katzmarzyk, P.T., Perusse, L., Rao, D.C., Bouchard,C. (2000). Familial risk of overweight and obesity in the Canadian population using the WHO/NIH criteria. Obes Res; 8: 194-197

[32]Koeppen-Schomerus, G., Wardle, J., Plomin, R. (2001). A genetic analysis of weight and overweight in 4-year-old twin pairs. Int J Obes Relat Metab Disord; 25: 838-844.

[33]Pietilainen, K.H., Kaprio, J., Rissanen,

A., Winter, T., Rimpela, A., Viken, R.J. et al (1999). Distribution and heritability of BMI in Finnish adolescents aged 16y and 17y: a study of 4884 twins and 2509 singletons. Int J Obes Relat Metab Disord; 23: 107-115.

[34]Fesinmeyer, M.D., North, K.E., Ritchie, M.D., Lim, U., Franceschini, N., Wilkens, L.R. et al. (2013).Ge- netic risk factors for BMI and obesity in an ethnically diverse population: results from the population architecture using genomics and epidemiology (PAGE) study. Obesity (Silver Spring); 21: 835-846

[35]Poulsen, P., Vaag, A., Kyvik, K., Beck-Nielsen, H. (2001). Genetic versus environmental aetiology of the metabolic syndrome among male and female twins. Diabetologia, 44:537-543.

[36]Wardle, J., Carnell, S., Haworth, C.M., Plomin, R. (2008). Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. Am J Clin Nutr, 87:398-404.

[37]Herrera B.M., Lindgren C.M. (2010). The Genetics of Obesity. Curr Diab Rep 10:498–505 DOI 10.1007/ s11892-010-0153-z.

[38]Moll, P.P., Burns, T.L., Lauer, R.M. (1991). The genetic and environmental sources of body mass index variability: the Muscatine Ponderosity Family Study. Am J Hum Genet, 49:1243-1255.

[39]Farooqi, I.S., Keogh, J.M., Yeo, G.S., Lank, E.J., Cheetham, T., O'Rahilly, S. (2003). Clinical spec- trum of obesity and mutations in the melanocortin 4 receptor gene. N Engl J Med. 20;348(12):1085-95.

[40]Bonnefond, A. et al., (2016). Eating Behavior, Low-Frequency Functional Mutations in the Melanocortin-4 Receptor (MC4R) Gene, and Outcomes of Bariatric Operations: A 6-Year Prospective Study. Diabetes Care; 39(8): 1384-1392.

[41]Lepsen, E.W., et al. (2018) Patients with Obesity Caused by Melanocortin-4 Receptor Mutations Can Be Treated with a Glucagon-like Peptide-1 Receptor Agonis. Cell Metabolism: 28, 23-32.

[42]Collet, T., et al. (2017). Evaluation of a melanocortin-4 receptor (MC4R) agonist (Setmelanotide) in MC4R deficiency. Molecular Metabolism Volume 6, Issue 10; 1321-1329.

Factors Affecting Catecholamines in Caregivers of Patients with Dementia

Akemi Hirano1* Yusuke Suzuki2 Toshio Hayashi3 Koichiro Ina4 Joji Onishi5

1.Department of Adult Nursing, Shubun University, 6 Nikko-cho, Ichinomiya, Aichi 491-0938, Japan

2.Centre for Community Liaison and Patient Consultations, Nagoya University Hospital, 65 Tsuruma, Showa, Nagoya, Aichi 466-8550, Japan

3.Department of Community and In-Home Nursing, Nagoya University Graduate School of Medicine, School of Health Sciences, Daiko-Minami, Higashi-ku, Nagoya, Aichi 461-8673, Japan

4.Department of Internal Medicine, Ina Clinic, 3-111 Hirabari, Tenpaku, Nagoya, Aichi 468-0011, Japan

5.Department of Community Healthcare & Geriatrics, Nagoya University Graduate School of Medicine, 65 Tsuruma, Showa, Nagoya, Aichi 466-8550, Japan

ABSTRACT

Background: Caregivers of dementia patients have significantly higher levels of serum IL-6 and CRP compared to non-caregivers, and the accumulation of everyday stressors reportedly promotes the induction of inflammatory markers. However, few studies have identified factors that affect catecholamine levels in caregivers who experience a combination of physical and mental stress from caregiving.Purpose: This study aimed to identify physical factors that impact catecholamine levels in caregivers of dementia patients. Methods: Participants were elderly caregivers living together with elderly Alzheimer's-type dementia patients. We performed logistic regression analysis, with levels of adrenaline, noradrenaline, and dopamine (indicators of catecholamine) as dependent variables. Results: Caregiver BMI had a significant impact on adrenaline levels (OR: 0.792; 95%CI: 0.654-0.960) and noradrenaline levels (OR: 1.210; 95%CI: 1.009-1.451), whereas age had a significant impact on dopamine levels (OR: 1.162; 95%CI: 1.019-1.324). Discussion: While caregiver BMI significantly impacted adrenaline and noradrenaline levels, the mechanism underlying these relationships is unclear. One possibility is that obesity (BMI) and a rise in sympathetic nerve activity contributed to hypertension. Our findings suggest that chronic stress in elderly caregivers may potentially impair the dopaminergic activation system in the brain. Conclusion: There is a need to identify factors which increase BMI in caregivers. Future studies aimed at gaining a better understanding of the lifestyle habits of caregivers and intervention studies aimed at reducing their BMI are warranted.

Keywords: Age;BMI;Caregiver burden;Catecholamine;Dementia

1.Introduction

The mortality risk of caregivers has been reported to be 63% higher than that of non-caregivers [1]. The chronic stress experienced by caregivers of dementia patients is thought to cause physiological changes in the body. Levels of D-dimer, a marker of fibrin formation and degradation, are reported to be significantly

higher in caregivers than in non-caregivers [2]. Similarly, caregivers of dementia patients have higher serum IL-6 and CRP levels relative to non-caregivers, and the accumulation of everyday stressors promotes an increase in inflammatory markers [3].

Patients with probable Alzheimer's type dementia have a median survival period of 11.3 years from onset and 5.7 years from diagnosis [4]. Caregivers of dementia patients are reported to experience a greater level of physical bur- den and mental distress than caregivers of patients with other disorders [5]. One in three caregivers experiences depression, with depression occurring more frequently in caregivers of dementia patients compared to caregivers of patients with other chronic diseases [6].

Aging and chronic stress can have a devastating im- pact on the vulnerable brain [7]. The various emotions resulting from stress are caused by neurotransmitters such as noradrenaline and dopamine. Pathways triggered by these neurotransmitters have been studied in detail and are known to be closely related to emotions [8]. Activation of sympathetic nerves results in the secretion of catecholamines (adrenaline, noradrenaline, and dopamine), leading to vasoconstriction, increased heart rate, and increased blood pressure, which can result in the onset of circulatory disorders. Moreover, mental stress is known to excite sympathetic nerves [9]. Many organs are innervated by autonomic nerves. For instance, autonomic nerves innervate endocrine grands and regulate hormone secretion.

Only a few studies have examined factors which impact catecholamine levels when caregivers of dementia patients suffer from a combination of physical and mental stress due to caregiving. Therefore, the present study aimed to identify health-related factors which impact catecholamine levels in caregivers of dementia patients.

2.Methods

2.1Participants

Participants were elderly caregivers living together with Alzheimer's-type dementia patients, and included caregivers who had good control of chronic diseases such as hypertension, diabetes, and dyslipidemia. Those with severe heart diseases and stroke were excluded.

2.2Research Design

This was a cross-sectional study conducted to analyze factors which influence catecholamines in caregivers.

2.3Caregiver Scales and Assessed Factors

To evaluate catecholamines, we assessed the levels of adrenaline, noradrenaline, and dopamine. Adrenaline has a heart-stimulating effect and is also involved in sugar and fat metabolism, noradrenaline has a hypertensive effect, and dopamine (a precursor of noradrenaline) has a specific effect on the central nervous system, renal system, circulatory system, and digestive system. The higher the levels of these catecholamines, the stronger their impact on the cardiovascular system.

The Japanese version of the Zarit Burden Interview (ZBI) was used to assess caregiver burden [10]. We also assessed BMI by caregivers.

2.4Ethical Considerations

This study was approved by the ethics committee of Nagoya University Graduate

School of Medicine. Participants provided informed consent after they received a clear explanation that participation in the present study was voluntary.

2.5Statistical Analysis

For logistic regression analysis, dichotomized (high or low) levels of adrenaline, noradrenaline, or dopamine based on median levels were entered into the models as dependent variables. Statistical analyses were performed using SPSS25. $P<0.05$ was considered statistically significant.

3.Results

Of the participants, 23 were male and 29 were female. Levels of noradrenaline and dopamine were slightly high- er than reference levels. Median systolic blood pressure values were 143.0 and 134.8 for males and females, respectively, with no significant difference between the two (P=0.574; Table 1).

Table 2 shows the results of the bivariate correlation matrix. Noradrenaline levels were significantly correlated with dopamine levels ($r=0.456$, $p<0.05$), but no significant correlation was observed between adrenaline and nor- adrenaline levels.

Table 3 shows the results of the bivariate correlation matrix for hormone secretion and caregiver factors. Adrenaline levels showed a significant negative correlation with BMI ($r=-0.345$, $P<0.05$), while noradrenaline levels showed a significant positive correlation with BMI ($r=0.297$, $P<0.05$). Dopamine levels showed a significant positive correlation with age ($r=0.354$, $P<0.05$).

Table 4 shows the results of a binomial logistic regression model with caregiver age, sex, total sense of care bur- den, BMI, and number of oral medications as independent variables. The high and low levels of each hormone were entered as dependent variables using the stepwise method. Hormone levels were dichotomized based on whether they were below or above the respective median level (0, 1) in order to extract factors that impact each hormone.

Caregiver BMI was extracted as a factor that significantly impacted adrenaline levels (OR: 0.792, 95%CI: 0.654-0.960) and noradrenaline levels (OR: 1.210, 95%CI: 1.009-1.451). Age was extracted as a factor that significantly impacted dopamine levels (OR: 1.162, 95%CI: 1.019-1.324). Binomial logistic regression analysis revealed that caregiver BMI significantly impacted.

4.Discussion

The present study identified age and BMI as health-related factors which impact catecholamine levels in care- givers of dementia patients.

Noradrenaline levels were found to be significantly correlated with dopamine levels. Stress is known to stimulate the hypothalamo pituitary-adrenal system, as well as the sympathoadrenal system [11-13]. Adrenaline is secreted into the blood from the adrenal medulla, noradrenaline is secreted from sympathetic nerve endings, and cortisol is secreted into the blood from the adrenal cortex [14-16]. Both noradrenaline and dopamine are secreted from the adrenal medulla. Noradrenaline and adrenaline have been report- ed to inhibit the production of

inflammatory cytokines by dendritic cells via β receptors [17-18]. However, little is known about their other effects. In the present study, noradrenaline and dopamine levels were correlated with each other, suggesting that both catecholamines may be an objective indicator of stress response, which we surmise reflects stress from caregiving.

Mental stress tests markedly induce epinephrine re- lease [19]. Epinephrine (adrenaline) responses are negative- ly correlated with changes in BMI and waist circumference [20]. Moreover, noradrenaline levels at rest have been shown to be a positive predictor of BMI [21]. Although we identified caregiver BMI as a significant factor which impacts adrenaline and noradrenaline levels, how they are related mechanistically remains unknown. Some of the impairments in plasma glucose-insulin homeostasis noted in visceral obesity may be related to an abnormal meta- bolic response to an adrenaline challenge [22]. According to one study, only plasma norepinephrine and BMI were significant independent predictors of blood pressure, suggesting that obesity and heightened sympathetic nervous system activity contribute to blood pressure elevation [23].

Age was identified as a factor which significantly impacts dopamine levels. Dopamine levels and dopamine transporter density have been reported to decrease with age[24-25]. However, our results are inconsistent with the report that dopamine transporter density declines with age. Dopamine levels were heightened in our participants, despite them being elderly with a median age of 76 years. In fact, we found that dopamine levels tended to increase with age, with dopamine and age showing a positive correlation. This suggests that dopamine levels are increased in the elderly caregivers of the present study. One reason for this observation might be that caregiving activities stimulated the brain, which in turn led to the activation of dopamine receptors.

Our findings suggest that neurotransmission is likely to be highly active in elderly caregivers. Furthermore, given that dopaminergic neurons are reportedly more susceptible to neuroinflammation from chronic stress than other types of brain cells[26-27], chronic stress in elderly caregivers might impair the dopaminergic activation system in the brain.

5.Conclusion

The present study identified factors which impact catecholamine secretion due to stress in caregivers of dementia patients. Specifically, age and BMI were found to impact catecholamine levels. In view of this finding, identifying factors which contribute to increased BMI will be important. Future studies aimed at gaining a better understanding of the lifestyle habits of caregivers and interventional studies aimed at reducing BMI are needed. Furthermore, the small sample size was another limitation of this study. Accordingly, future studies should follow and observe these blood biochemical factors in the long term.

Acknowledgements

We thank all participants for their cooperation with the study.

Disclosure statement

The authors declare no conflict of interest.

Table 1. Participant characteristics

	Median	IQR (25-75%)
Caregiver Age	76	72-79
Adrenaline (Ref: ≤100) (pg/mL)	39.5	22.3-56
Noradrenaline (Ref: 100-450) (pg/mL)	610	471-718.3
Dopamine (Ref: ≤20) (pg/mL)	21.0	14-31.3
BMI	23.4	20.7-25.2
SBP	142	125.5-147.8
DBP	78	72.8-87.5

BMI: Body mass Index, SBP: systolic blood pressure, DBP: diastolic blood pressure

Table 2. Associations of outcome measures

		1	2	3
1. Adrenaline	r	1.000		
p (two-tailed)				
2. Noradrenaline	r	.137	1.000	
p (two-tailed)		.333		
3. Dopamine	r	.188	.456*	1.000
p (two-tailed)		.183	.001	

r: Spearman's correlation coefficient; p: significance level, *p < 0.05

Table 3. Correlations between catecholamines and care- giver factors

		Age	SBP	BMI
Adrenaline	r	.099	-.002	-.345*
	P	.487	.988	.012
Noradrenaline	r	.002	.030	.297*
	P	.991	.832	.032
Dopamine	r	.354*	-.066	-.069
	P	.010	.640	.625

r: Spearman's correlation coefficient; p: significance level, *p < 0.05

Table 4. Logistic regression analysis with catecholamines as the dependent variable

					95% CI	
Dependent variable	Variable (covariate)	B	P	OR	Lower limit	Upper limit
Adrenaline	BMI	-.233	.017	.792	.654	.960
Noradrenaline	BMI	.191	.040	1.210	1.009	1.451
Dopamine	Caregiver age	.150	.025	1.162	1.019	1.324

Two groups for each catecholamines based on median values were created, as follows: adrenaline (<39.5=0;≥39.5=1), noradrenaline (<610=0; ≥610=1), dopamine (<21=0; ≥21=1). B: partial regression coefficient, P: level of significance, 95%CI: 95% confidence interval, BMI: Body Mass Index.

References

[1]Schulz R, Beach SR. Caregiving as a risk factor for mortality: the Caregiver. Health Effects Study, JAMA, 1999, 282:2215-2219.DOI:10.1001/jama.282.23.2215

[2]von Kanel R, Dimsdale JE, Adler KA, Patterson TL, Mills PJ, Grant I. Exaggerated plasma fibrin formation (D-dimer) in elderly Alzheimer caregivers as compared to noncaregiving controls. Gerontology, 2005, 51:7-13. DOI:10.1159/000081428

[3]Gouin JP, Glaser R, Malarkey WB, Beversdorf D,

Kiecolt-Glaser J. Chronic stress, daily stressors, and circulating inflammatory markers. Health Psychol, 2012, 31:264-268.
[4]Waring SC, Doody RS, Pavlik VN, Massman PJ, Chan W. Survival among patients with dementia from a large multi-ethnic population. Alzheimer Dis Assoc Disord, 2005, 19:178-183. DOI:10.1097/01.wad.0000189033.35579.2d
[5]Kim Y, Schulz R. Family caregivers' strains: comparative analysis of cancer caregiving with dementia, diabetes, and frail elderly caregiving. J Aging Health, 2008, 20:483-503.
DOI: 10.1177/0898264308317533
[6]Schoenmakers B, Buntinx F, Delepeleire J. Factors determining the impact of care-giving on caregivers of elderly patients with dementia. A systematic literature review. Maturitas, 2010, 66(2):191-200. DOI:10.1016/j.maturitas.2010.02.009
[7]Prenderville JA, Kennedy PJ, Dinan TG, Cryan JF. Adding fuel to the fire: the impact of stress on the ageing brain. Trends Neurosci, 2015, 38(1):13-25.DOI:10.1016/j.tins.2014.11.001
[8]Jung YH, Shin NY, Jang JH, et al. Relationships among stress, emotional intelligence, cognitive intelligence, and cytokines. Medicine (Baltimore) 2019, 98:e15345. DOI:10.1097/MD.0000000000015345
[9]Ziegler MG, Milic M. Sympathetic nerves and hypertension in stress, sleep apnea, and caregiving. Curr Opin Nephrol Hypertens. 2017, 26(1):26-30.DOI:10.1097/MNH.0000000000000288
[10]Zarit SH, Reever KE, Bach-Peterson J. Relatives of the impaired elderly: correlates of feelings of burden. Gerontologist 1980; 20: 649–655.
[11]Brown MR, Fisher LA. 1.E41. Brain peptide regulation of adrenal epinephrine secretion. Am J Physiol. 1984, 247(1 Pt 1):E41-6.DOI:10.1152/ajpendo.1984.247
[12]Udelsman R, Goldstein DS, Loriaux DL, Chrousos GP. Catecholamine-glucocorticoid interactions during surgical stress. J Surg Res, 1987, 43(6): 539-45. DOI:10.1016/0022-4804(87)90128-4
[13]Naito Y, Fukata J, Tamai S, Seo N, Nakai Y, Mori K, Imura H. Biphasic changes in hypothalamo-pitu-itary-adrenal function during the early recovery period after major abdominal surgery. J Clin Endocrinol Metab, 1991, 73(1):111-7.DOI:10.1210/jcem-73-1-111
[14]Weissman C. The metabolic response to stress: an overview and update. Anesthesiology, 1990, 73(2):308-27.DOI:10.1097/00000542-199008000-00020
[15]Halter JB, Pflug AE, Porte D Jr. Mechanism of plas- ma catecholamine increases during surgical stress in man. J Clin Endocrinol Metab, 1977, 45(5):936-44. DOI:10.1210/jcem-45-5-936
[16]Esler M. Assessment of sympathetic nervous function in humans from noradrenaline plasma kinetics. Clin Sci (Lond). 1982, 62(3):247-54.DOI:10.1042/cs0620247
[17]Goyarts E, Matsui M, Mammone T, Bender AM, Wagner JA, Maes D, Granstein RD. Norepinephrine modulates human dendritic cell activation by altering cytokine release. Exp Dermatol, 2008, 17(3):188-96. DOI:10.1111/j.1600-0625.2007.00677.x
[18]Maestroni GJ. Short exposure of maturing, bone marrow-derived dendritic cells to norepinephrine: impact on kinetics of cytokine production and The develop- ment. J Neuroimmunol. 2002, 129(1-2):106-14.DOI: 10.1016/s0165-5728(02)00188-1
[19]LeBlanc J, Côté J, Jobin M, Labrie A. Plasma cate- cholamines and cardiovascular responses to cold and mental activity. J Appl Physiol Respir Environ Exerc Physiol, 1979, 47(6):1207-11.DOI:10.1152/jappl.1979.47.6.1207
[20]Flaa A, Sandvik L, Kjeldsen SE, Eide IK, Rostrup M. Does sympathoadrenal activity predict changes in body fat? An 18-y follow-up study. Am J Clin Nutr, 2008, 87(6):1596-601.DOI:10.1093/ajcn/87.6.1596
[21]Masuo K, Kawaguchi H, Mikami H, Ogihara T, Tuck ML. Serum uric acid and plasma norepinephrine concentrations predict subsequent weight gain and blood pressure elevation. Hypertension. 2003, 42(4): 474-80. DOI:10.1161/01.HYP.0000091371.53502.D3
[22]Mauriège P, Klein Kranenbarg WM, Prud'homme D, Lamarche B, Tremblay A, Bouchard C, Nadeau A, Després JP. Insulin and glucagon responses to adren- aline infusion in abdominal obese men. Int J Obes Relat Metab Disord, 1996, 20(7):668-76.
[23]Masuo K, Mikami H, Itoh M, Ogihara T, Tuck ML. Sympathetic activity and body mass index contribute to blood pressure levels. Hypertens Res. 2000, 23(4):303-10. DOI:10.1291/hypres.23.303
[24]Volkow ND, Logan J, Fowler JS, GJ. Wang, Gur RC,C. Wang, Felder C, Gatley SJ, YS. Ding, Hitzemann R, Pappas N. Association between age-related decline in brain dopamine activity and impairment in frontal and cingulate metabolism. Am J Psychiatry. 2000, 157(1):75-80.
DOI:10.1176/ajp.157.1.75
[25]Lavalaye J, Booij J, Reneman L, Habraken JB,

van Royen EA. Effect of age and gender on dopamine transporter imaging with [123I]FP-CIT SPET in healthy volunteers. Eur J Nucl Med. 2000, 27(7): 867-9. DOI:10.1007/s002590000279
[26]Block ML, Hong JS. Chronic microglial activation and progressive dopaminergic neurotoxicity. Biochem Soc Trans, 2007, 35(Pt 5):1127-32.DOI:10.1042/BST0351127
[27]HM. Gao, B. Liu, JS. Hong. Critical role for microglial NADPH oxidase in rotenone-
induced degeneration of dopaminergic neurons. J Neurosci, 2003, 16, 23(15):6181-7.DOI:10.1523/JNEUROSCI.23-15-06181.2003

Author's contributions

Akemi Hirano conceived the idea and designed the study. Akemi Hirano carried out the data analysis and interpretation. Akemi Hirano wrote the first draft of the manuscript and Yusuke Suzuki, Koichiro Ina, Joji Onishi critically discussed all versions of the manuscript. Yusuke Suzuki, Koichiro Ina, and Toshio Hayashi recruited the participants, and Akemi Hirano contributed to the overall supervision of the study.

The Gulf Stream and the Californian Current as Factors Affecting the Behavior and Health of Americans

Yuri Pivovarenko*
Research and Training Center 'Physical and Chemical Materials Science' Under Kyiv Taras Shevchenko University and NAS of Ukraine, Kiev, Ukraine

ABSTRACT

Due to the existence of the Earth's geomagnetic field, Lorentz's forces constantly act on all sea currents. These forces distribute the charges of sea currents in both vertical and horizontal directions. In particular, this distribution manifests itself in the electric polarization of sea currents in directions perpendicular to them. So, earlier it was shown that the same Lorentz forces cause negative electrization of the Sargasso Sea. It is also shown here that the positive electrization of the western edge of the Gulf Stream and, consequently, the eastern coast of the United States is also caused by the Lorentz force arising from the interaction of this sea current with the vertical component of the geomagnetic field. It is also shown here that the positive electrization of east edge of California Current together with west coast of USA is also caused due to the similar reasons. All this allows us to conclude that an increased concentration of positive air ions is constantly retained in the air both in the east and in the west of the United States. This situation has caused the need for an analysis of how the predominantly positive electrization of the air affects both human health and their physical and mental activity. The results of this analysis are presented here. It is also shown that these results can be useful for residents of some other countries.

Keywords: Environment;Electrization;Gulfstream;Metabolism;Stroke;Thrombogenesis;Alzheimer 's disease;Viral infections;Feng Shui

1.Introduction

It is well known that the Lorentz force FL acts on charges moving in a magnetic field:FL = q[v, B](1)where: q – an electric charge moving in a magnetic field;v – the speed of movement of such a charge q; B – magnetic field induction [1,2].

This allows us to conclude that the earth's surface, including water, which continuously crosses the lines of force of the geomagnetic field during its own diurnal rotation (Figure 1), is constantly exposed to the Lorentz forces,which continuously separate positive and negative earthly charges, both in vertical and horizontal directions [3-5]. This separation occurs even more effectively in air and sea currents, which are subjected to the action of additional Lorentz forces arising from the movement of these currents relative to the vertical component of the geomagnetic field [4,5].

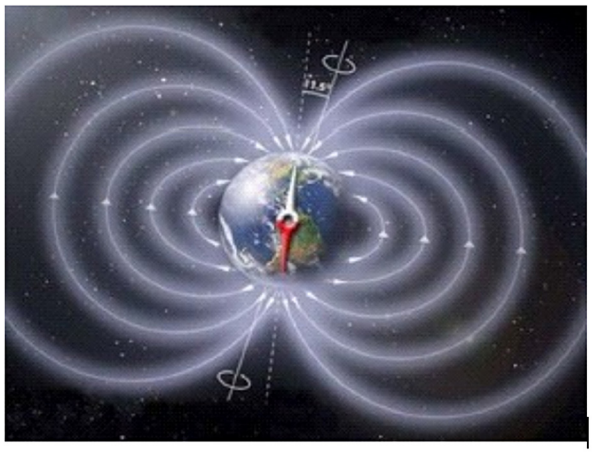


Figure 1. Since the Earth rotates around its own axis, all objects located on the earth's surface constantly intersect the lines of force of the geomagnetic field [3-5].

Thus, as a result of the interaction of the clockwise waters of the Sargasso Sea (Figure 2) with the vertical component of the geomagnetic field, directed downward in the northern hemisphere of the Earth, negative charges are concentrated inside this sea and positive charges – at its periphery [5] (in fact, as a result Hall's effect [1]).

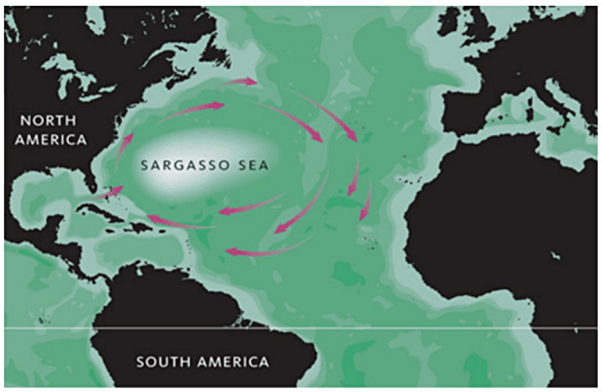


Figure 2. The Sargasso Sea is located in the Northern hemisphere of the Earth and is limited by currents moving clockwise. Equator is marked with a white horizontal line [5].

Thus, as a quite expected result of the interaction of the Gulf Stream (Figure 3, red arrows to the right of North America) with the vertical component of the geomagnetic field, which is directed downward in the northern hemisphere of the Earth, is that positive charges are concentrat ed on the western side of this sea current (resulting in the same Hall's effect [1]

For this reason, the land, water and air of the US East Coast is constantly saturated with positive ions, mainly hydrated protons, which most actively evaporate from the surface of positively charged water [3-5]

Moreover, as a result of the interaction of the California Current (Figure 3, downward blue arrow to the left of North America) with the same vertical component of the geomagnetic field, positive charges are concentrated on its eastern side. For this reason, the land, water and air of the US west coast are saturated with positive ions at least constantly.

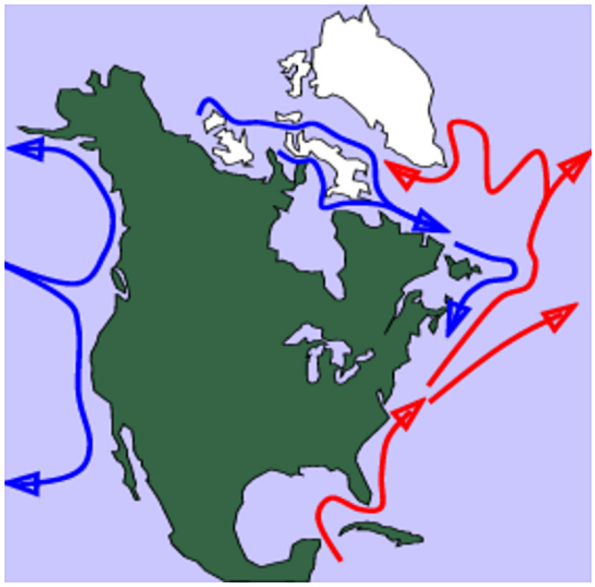


Figure 3. Near North America, there is the Gulf Stream (red arrows) and the California Current (blue arrow point ing down, to the left of the continent).

While important for completeness, it should also be noted that the same positive electrization occurs on the northern coast of the Gulf of Mexico and the east and west coasts of Florida (Figure 3); of course, this electrization is the result of the interaction of the nascent Gulf Stream with the same vertical component of the geomagnetic field.

Thus, people who live on all coasts of the mainland United States, with the exception of Alaska, are constantly under the influence of an all-encompassing positive electrization. Let us discuss how this electrization determines the characteristics of the nervous and physical activity of such people, as well as how it affects their health.

To attract the attention of a larger audience, it should be added that the same reasons for the discussed positive electrization, which were mentioned, exist in other regions of the Earth. So, the sea currents are exist of the east coasts of Japan and Brazil, as well as off the southeast coast of Australia, also charge them positively. (It should be noted that the last two currents are directed from north to south and that the vertical component of the geomagnetic field is directed upwards in the southern hemisphere of the Earth, where both these countries are located.) Thus, the phenomena discussed here have a planetary distribution.

2.Discussion

First of all, the fact that glucose is transported through the cytoplasmic membranes by means of a symport (Figure 4), the intensity of which is determined by the concentration of extracellular protons, which are directly involved in the creation of the “proton drawing force” (pdf) [6-8], musts be considered. Since glucose is the main "fuel" of nerve and muscle cells, at least the rate of its transport through their outer membranes determines their functional activity and, consequently, the nervous and muscular activity of people in general. Already this transport function of protons suggests that humans are very active in environments that are saturated with them.

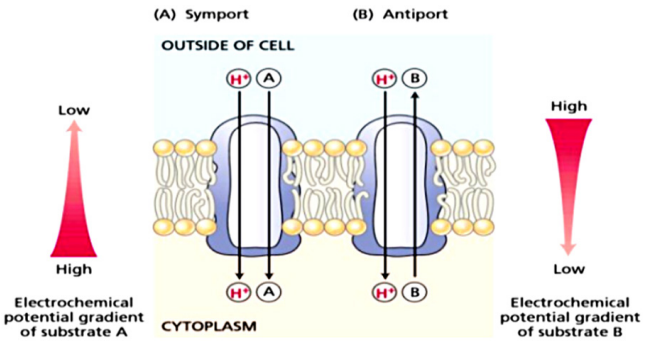


Figure 4. The energy of proton gradients on cytoplasmic membranes allows cells to realize two types of secondary active transport: symport and antiport. At the symptom (A), a proton, penetrating into a cell from the outside, captures one glucose molecule. With antiport (B), the energy "scattered" by a proton entering the cell from the outside can be used to remove cations (for example, sodium ions) from the cell [8].

Moreover, the positive electrization of the environment can cause an increase in the tone of the human body as a whole and, in particular, of its skin and blood vessels. This possibility is due to the fact that positive electrization of water (which is the main component of the human body) increases its surface tension and, as a result, causes its compression, in contrast to

negative electrization of water (Figure 5) [9]

It is also appropriate to recall that the head of a standing or sitting person is the most positively charged part of his body [11] This means that the human brain is evolutionary turned to positive electrization, which increases its metabolism and, therefore, both its activity and development.

Due to the small surface tension, water with negative electric potential can spread throughout the bottom of the Petri dish; due to the large surface tension, water with a positive electric potential cannot spread throughout the bottom of the Petri dish.

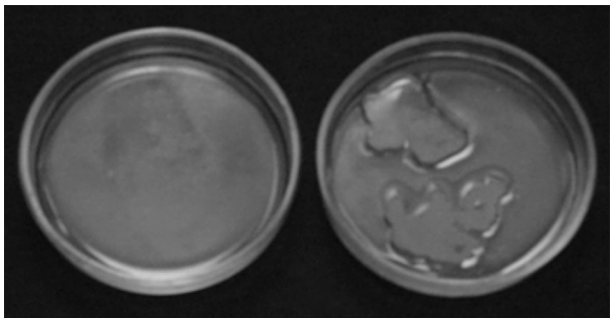


Figure 5. Left: 5 ml of water with a potential of –200 mV completely cover the bottom of the Petri dish. Right: 5 ml of water with a potential of +200 mV do not completely cover the bottom of a Petri dish. Both water used had 20 – 22 °C [5,9].

Given, in addition, the positive electrization of the air stimulates the transfer of essential nutrients from the intestine to the blood [12], one can conclude that the constant saturation of the ambient air with positive air ions and, therefore, hydrated protons determines the permanent physical and intellectual activity of most Americans.

Unfortunately, this activity also has negative manifestations, which include the permanent aggressiveness of the Americans, which manifests itself in both high crime and high accident rates on the roads. Moreover, the constantly high tone of permanently positively charged blood vessels can initiate their destruction and hence bleeding. Starch powder applied to the surface of positively charged water makes it possible to convincingly demonstrate its "destructive" ability: as you can see, positively charged water literally "breaks" a lump of starch powder applied to its surface, unlike negatively charged water (Figure 6).

Figure 6. Left: the starch powder covers the surface of the water with potential +250 mV practically wholly. Right: powder starch remains in the same place where it was put in water potential –200 mV. Both water used had 20 – 22°C [5,9].

Moreover, the "bursting" ability of positively charged water is confirmed by the fact that it destroys the films formed during the drying of collagen solutions prepared in such water (Figure 7); besides, it should be born in mind that negatively charged water does not demonstrate such destructive power, at least in relation to collagen.

Figure 7. This is a "cracked" film, into which the drying collagen solution, prepared in water with a potential of+250 mV, has turned. Water used had 20 – 22 °C.

Thus, bearing in mind that elastin, which is a structural analogue of collagen, is the main structural protein of blood vessels [13], the obtained result (Figure 7) suggests that positive electrization can be the main cause of non-traumatic destruction of blood vessel walls, in fact, the main cause of stroke. The fact that only positively charged water is capable of causing swelling of various biopolymers [9] must be also be taken into consideration, because it suggests that the same positive electrization stimulates the formation of blood clots, i.e., thrombus formation. (Probably, this thrombus formation is a defense reaction of the body to the destruction of the walls of the blood vessels. In any case, such coordination looks quite natural, since it allows you to stop bleeding through the ruptured walls of the blood vessel.)

Unfortunately, these are far from all the discussed harmful effects of the discussed positive electrization. So, it is likely that it contributes to the spread of viral infections. To better understand how this might happen, one must first understand the general importance of positive electrization of DNA molecules (together with their immediate environment) for their successful introduction into cells undergoing artificial genetic modification. The need for this electrization is becoming almost obvious if to take into account the efficiency of using cationic (exclusively!) polymers for introducing DNA molecules into target cells, as well as the need for positive electrization in such methods of genetic modification of cells as DE- AE-dextrin method and lipofection method. Since this is relevant right now, it should be added right away that all these methods are also effective for the introduction of RNA to target cells [14]

To better understand this very need for positive electrization of introduced DNAs more fully, it is necessary to analyze the phenomena underlying the cryogenic method of genetic transformation of bacterial cells, which consists in preliminary deep cooling of a mixture containing recipient cells and injected DNA, followed by heating this mixture to 37 – 42 °C [15] Since this is necessary, let's remember a Kyon's rule right now: when two phases are in contact, the phase with a higher dielectric permittivity receives a positive charge and the phase with a lower – negative [10,16]. Since the dielectric constant of water is ~73.1 at 40 °C and 88.3 at 0 °C [10], a cold water mixture accumulates protons and, therefore, acquires a positive charge, and warm water in contact with it loses protons and, accordingly, acquires a negative charge, naturally – in accordance with the aforementioned Kyon's rule. As you can see, the cryogenic method of genetic transformation of cells is also based on the formation of an electrical gra- dient, most likely a stepwise proton gradient on the outer

membranes of target cells. This suggests that the transfer of DNA molecules into modified cells occurs together with a flux of protons directed from a warmer aqueous phase to a colder one, thus striving to create a charge dis- tribution provided for by the Kyon's rule [10,16]

All these examples should convince that the transport function of protons extends not only to the relatively small molecules and ions (Figure 4), but also to large. Accord- ingly, this suggests that such a function of protons is universal. Besides, all this suggests that positive electrization of the human body via to the correspondingly electrized environment can increase a human susceptibility to viral infections, naturally assuming that the extrapolation of these phenomena to human cells is correct.

3.Conclusions

To make these conclusions easier to accept, initially compare how you feel in bright and cloudy weather, given at the same time that clear weather usually coincides with positive electrization of the lower atmosphere, and cloudy weather – their negative electrization [17]. Moreover, you can compare how you feel during the day and at night, given the increased positive electrization of the daytime side of the Earth [18]. It is likely that all these comparisons can definitively convince you that the variations in air electrization are natural, as well as that they affect people.

Besides, these comparisons will allow us to agree that the targeted electrization of ambient air allows you to con troll the well-being and behavior of people. Thus, it can be assumed that the targeted negative electrization of the air will reduce the levels of crime and road accidents, as well as the incidence of stroke and unwanted thrombosis. It can also be hoped that the same negative electrization can prevent the ingress of foreign nucleic acids, including viral ones, into human cells, preventing the spread of viral infections in general. If we add that negative electrization of public buildings and vehicles can prevent many bacterial infections [19], then its purposeful use would seem more than reasonable. (The fact that such negative electrization can also prevent cancer [20] also speaks in its favor.)

Moreover, any electrization must be sufficiently justified. So, the fact that the brain is abnormally compressed in Alzheimer's patients [21] suggests that this contraction is due to positive electrization, just like the compression of positively charged water (Figure 6, right). Accordingly, it can be assumed that negative electrization of the air surrounding patients with Alzheimer's disease can be transmitted to their brains, causing the same decompression as in the case of negative electrization of water (Figure 6, left). At the same time, one should not ignore the idea that impairment of glucose transport into neuronal cells is the true cause of Alzheimer's disease [21]. Therefore, this idea suggests that positive electrization of the brain of patients will stimulate glucose symport through the outer membranes of brain cells, in accordance with the scheme shown in Figure 4, A. Thus, according to this idea, it is precisely positive electrization of air that may not only be beneficial, but also vital for Alzheimer's patients. (At the same time, one should not forget that the

human brain consists mainly of water, where its content is estimated at ~ 80%, and also that the human brain is located next to the respiratory tract.)

At all events, it is necessary to consider all the possible consequences of any electrization of the air. It should be noted that this consideration can be very useful for both climatologists and balneologists using the effect of natural factors on patients. In addition, the same consideration can be no less useful for Feng Shui adherents who seek to use the effect of air and water currents on people, both natural and artificial. In any case, all of them can now consciously use the fact that the direction of rotation of air or water determines the sign of their electrization, which can be negative, as in the Sargasso Sea (Figure 2), or positive, as on the ocean coasts of the United States (Figure 3). In par ticular, it should be taken into account that it is convenient to obtain the desired electrization of the air with the help of appropriately oriented fans, especially since the effectiveness of this type of electrization is confirmed by visual experiments [20,22].

References

[1]Purcell, E.M., Morin, D.J., 2013. Electricity and Magnetism in BPC, 3rd Edition. Cambridge: Cam- bridge University Press. pp. 853.

[2]Feynman R., Leighton R., Sands M., 1965. FLP, 2.Moscow: Mir. pp. 166. In Russian.

[3]Pivovarenko, Y., 2018. The Nature of the Celestial Elves, Sprites and Jets. Discovery Nature. 12, 1-4.

[4]Pivovarenko, Y., 2019. Earth's Electromagnetic Forces and Their Participation in the Creation of Tornadoes. American Journal of Electromagnetics and Applications. 7(1), 8-12.

[5]Pivovarenko, Y., 2020. Negative Electrization of the Sargasso Sea as the Cause of Its Anomaly. American Journal of Electromagnetics and Applications. 8(2), 33-39.

[6]Lane, N., 2010. Why Are Cells Powered by Proton Gradients? Nature Education. 3(9), 18.

[7]Lane, N., Allen, J.F., Martin, W., 2010. How did LUCA make a living? Chemiosmosis in the origin of life. Bioassays. 32, 271-280.

[8]Taiz, L., Zeiger, E., 2002. Plant Physiology, 3rd ed. Sunderland (UK): Sinauer Associates, Inc. pp. 690.

[9]Pivovarenko, Y., 2018. The Electric Potential of the Female Body Liquids and the Effectiveness of Cloning. Research and Reviews on Healthcare: Open Access Journal, Lupine Publishers, LLC. 1(2), 22-26.

[10]Nekrasov, B.V., 1974. Bases of General Chemistry, Vol. 1. Moscow: Chemistry. pp. 656. In Russian.

[11]Pivovarenko, Y., 2020. The Use of Electromagnetic Forces of the Earth in Manual and Physiotherapy. Journal of Human Physiology. 2(1), 10-15.

[12]Pivovarenko, Y., 2019. Biochemical and Physiological Basis for Treating Hydrogen Gas as a Medicine. European Journal of Preventive Medicine. 7(6), 100- 107.

[13]Xu, J., Shi, G.P., 2014. Vascular wall extracellular matrix proteins and vascular diseases. Biochim Bio- phys Acta. 1842(11), 2106-2119.

[14]Shirokova, O.M., Vedunova, M.V., 2013. Methods of Genetic Transformation. Nizhny Novgorod: Nizhny Novgorod University Publishing House. pp. 30. In Russian.

[15]Lysak, V.V., 2005. Microbiology. Minsk: Edition of Belarus State University. pp. 261. In Russian.

[16]Voyutsky, S.S., 1964. The course of colloid chemistry. Moscow: Chemistry. pp. 574. In Russian.

[17]Kuznetsov, V.V., Cherneva, N.I., Druzhin, G.I., 2007. On the Influence of Cyclones on the Atmospheric Electric Field of Kamchatka. Reports of the Academy of Sciences. 412(4), 1-5.

[18]Pivovarenko, Y., 2017. The Electrical Polarization of the Earth in Its Orbital Motion. World Journal of Ap- plied Physics., 2(4), 97-100.

[19]Shepherd, S.J., Beggs, C.B., Smith, C.F., Kerr, K., 2010. Effect of negative air ions on the potential for bacterial contamination of plastic medical equipment. BMC Infectious Diseases. 10, 92.

[20]Pivovarenko, Y., 2021. Electrized Water as a Regu- lator of Cell Proliferation. Journal of Oncology Re-

search. 3(1), 1-10.

[21]Stasevich, K., 2017. Shimmering Hope: Can Alzhei-mer's disease be cured with light? Science and Life. 1, 44-48. In Russian.

[22]Pivovarenko, Y., 2019. Earth's Electromagnetic Forces and Their Participation in the Creation of Tornadoes. American Journal of Electromagnetics and Applications. 7(1), 8-12.

Importance and Risk Prediction of ABO Blood Group and Rh Factor in Papillary Thyroid Cancer

Dilek Geneş* Zafer Pekkolay Mehmet Şimşek Hüsna SaraçoğluMehmet Turgut Alpaslan Kemal Tuzcu
Dicle University Faculty of Medicine, Department of Adult Endocrinology and Metabolism, Diyarbakir, 21090, Turkey

ABSTRACT

Objective: There are limited data in the literature regarding the potential relationship between thyroid cancer and ABO blood types and Rh factor. The aim of our study was to investigate whether papillary thyroid cancer (PTC) is associated with blood type.Materials and Methods: The present study included patients who presented to Dicle University Faculty of Medicine between June 2009 and December 2020 and were diagnosed with PTC as a result of postoperative (thyroidectomy) histopathological analysis. The control group consisted of individuals whose blood type was analyzed at a random blood center.Results: Of the 223 patients diagnosed with PTC, 163 (73.1%) were females and 60 (26.9%) were males. In the comparison of patients based on ABO blood types and Rh factor, A Rh positive blood type was found 31% less frequently in the PTC group compared with the control group, and thus it was associated with a lower risk of PTC (OR:0.69; 95% Confidence Interval: 0.50–0.96, p=0.029).Conclusions: In our study, we found A Rh positive blood type to be significantly less frequent among patients with PTC. A Rh positive blood type can be considered as a protective factor indicating a reduced risk of PTC

Keywords: Papillary;thyroid cancer;Blood types;ABO;Rh

1.Introduction

Thyroid cancers are the most common endocrine malignancy and account for approximately 3% of the global cancer incidence [1]. Differentiated thyroid cancers (DTCs) arising from thyroid follicular epithelial cells account for the vast majority of thyroid cancers. A total of 3%–9% of DTCs are familial [2], and approximately 85% of these DTCs are papillary thyroid cancers (PTCs) [3]. Increased incidence rates are mainly due to increased PTC diagnosis rates. Detection of small and subclinical PTCs has become easier with the improvements in imaging techniques, biopsy methods (fine needle aspiration biopsy), medical surveillance, and healthcare accessibility [4]. Therefore, the incidence of thyroid cancer is still increasing [5].

The well-known risk factor for the development of thyroid cancers, particularly PTC, is exposure to ionizing radiation, particularly during childhood [2,6-9]; however, it is considered that other factors (family history, sex, obesity, smoking, alcohol consumption, hormonal exposure, and some environmental pollutants) may also play a role [1,9,10]. It is

believed that exposure to certain chemicals during intrauterine life and early childhood, especially along with possible epigenetic changes, may result in a mutagenic tendency in thyroid cells [6]. An inverse relationship between smoking and alcohol consumption and thyroid cancer has been mentioned in some studies [9,10]. Other factors that are believed to cause thyroid cancer include high dietary iodine content (especially PTC) [8], as well as intakes of selenium, goitrogens, and carcinogens. High thyroid stimulating hormone levels and genetic syndromes (Gardner, Cowden, and Werner syndromes) have been associated with DTC [7]. Because most flame retardants have chemical structures similar to thyroid hormones, they have been reported to alter thyroid hormone homeostasis and have ultimately become one of the suggested causes owing to their potential effects on the risk of thyroid cancer [10].

Blood type analysis is a key procedure in the blood transfusion process along with genetic analyses and associated disease examinations. The first blood type antigen system was the ABO system [11]. ABO blood type antigens are defined by the carbohydrate moieties on the outer surface of the erythrocyte cell membrane [12]. ABO antigens are also secreted from many sites other than erythrocytes, including platelets, vascular endothelial cells, mucous, and epithelial tissues [13]. The Rh system is the second most important blood type system in the preliminary transfusion test [11].

Clinical studies have shown that the ABO blood type plays a role in various diseases and malignancies [14]. The relationship between ABO blood types and cancer was first reported in 1953. Since then, numerous studies have been published with often conflicting results [13]. Although some studies evince a relationship between ABO blood type antigens and various cancer types, there is limited data on its prognostic significance in patients [12]. Further large-scale studies are warranted to determine whether ABO antigens have a function and, if they do, how this function contributes to tumorigenesis [13]. In this study, we aimed to determine whether PTC, a type of DTC, is associated with blood type.

2.Materials and Methods

Patients who presented to Dicle University Faculty of Medicine between June 2009 and December 2020 and were diagnosed with PTC as a result of postoperative (thyroidectomy) histopathological analysis and a control group consisting of individuals whose blood type was analyzed at a random blood center were retrospectively included in the present study.

The control group consists of people who applied to the emergency department of our hospital between these dates for any reason and whose blood groups were studied in the blood center. Individuals were selected by simple randomization to avoid bias from the hospital system. No disease screening was performed in the control group. Adults older than 16 years were included in the control group. It is known that the prevalence of thyroid diseases is higher in female gender. Therefore, gender matching was not done between the groups so that the control group represents the real population.

The blood types of these individuals were recorded. ABO and Rh blood types were

determined at the blood center using the gel centrifugation method (column agglutination; Ortho AUTOVUE INNOVA). Individuals aged <16 years, patients with concomitant non-thyroid malignancies, and those with other non-PTC thyroid ma- lignancies were excluded from our study. This study was approved by Dicle University medical ethics committee (No: 145/2021) in accordance with the ethical standards of the Declaration of Helsinki.

The data were analyzed using SPSS Version 22. The control and PTC groups were compared. Chi-square test was used to compare categorical variables and Mann-Whitney U test was used to compare numerical variables between groups. For statistical significance, results with $p<0.05$ at 95% Confidence Interval (CI) were considered significant.

3.Results

Of the 223 patients with PTC in the study, 163 (73.1%) were females and 60 (26.9%) were males. Their median age (minimum–maximum) was 43 (16–86) years. Of the 1040 individuals in the control group, 547 (52.6%) were females and 493 (47.4%) were males. Their median age (minimum–maximum) was also 43 (16–90) years. PTC was observed at a significantly higher rate in females ($p<0.001$). A comparison based on ABO blood types and Rh factor revealed that A Rh positive blood type was ob- served at a lower rate in the PTC group than in the control group (OR:0.69; 95% CI: 0.50–0.96, p=0.029). However,there were no significant differences among other blood types and Rh factors. The occurrence rates of blood types in the PTC and control groups and the demographic characteristics of the individuals are presented in Table 1.

Although A blood type was the most common blood type in Diyarbakir, Turkey (control group), with an occurrence rate of 37.6%, O blood type was found to be the most common blood type in the PTC group, with an occurrence rate of 38.6%. Percentage distributions of blood types are presented in Figure 1. In Figure 2, the distributions of ABO blood types and Rh factor in the PTC and control groups are presented. A Rh positive blood type was found at an occurrence rate of 33.6% in the control group and 26% in the PTC group, whereas O Rh positive blood type was found at an occurrence rate of 32.1% in the control group and 35% in the PTC group.

Table 1. Distribution of ABO blood types and Rh factor in the control and papillary thyroid cancer groups

	PTC n = 223(%)	Control n = 1040(%)	p value
Age (years)*	43 (16 -86)	43 (16 -90)	0.261
Sex Female Male	163 (73.1%) 60 (26.9%)	547 (52.6%) 493 (47.4%)	<0.001 <0.001
ABO			
A Positive	58 (26%)	349 (33.6%)	0.029
A Negative	10 (4.5%)	42 (4%)	0.761
B Positive	40 (17.9%)	172 (16.5%)	0.612
B Negative	10 (4.5%)	27 (2.6%)	0.129
AB Positive	16 (7.2%)	74 (7.1%)	0.975
AB Negative	3 (1.3%)	12 (1.2%)	0.811
O Positive	78 (35%)	334 (32.1%)	0.408
O Negative	8 (3.6%)	30 (2.9%)	0.577
Rh factor			
Rh Positive	192 (86.1%)	929(89.%)	0.229
Rh Negative	31 (13.9%)	111 (10.7%)	0.575

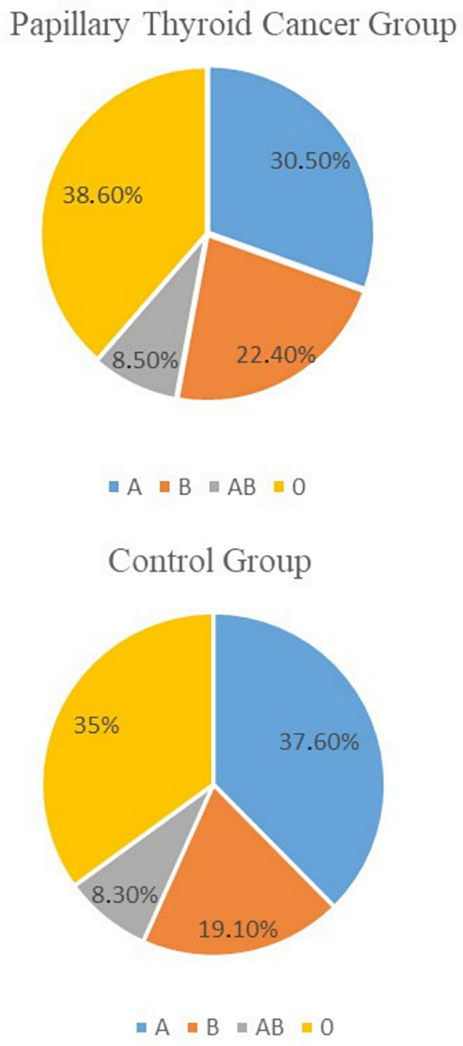


Figure 1. Schematic distribution of ABO blood types

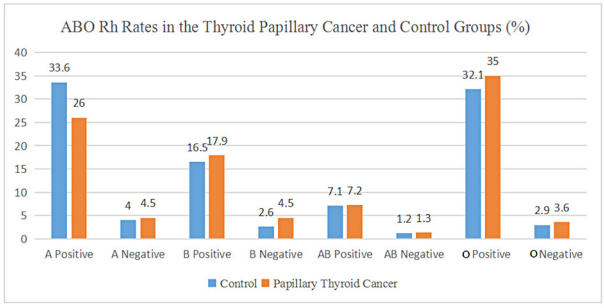


Figure 2. Occurrence rates of ABO blood types and Rh factor in the thyroid papillary cancer and control groups (in percentages)

4.Discussion

Over 90% of thyroid cancers are of the follicular or papillary variants often termed differentiated thyroid cancer [15]. Many factors other than ionizing radiation have been reported to be associated with the etiology of thyroid cancer, but a definite causality has not yet been established. There are limited data in the literature regarding the possible relationship between thyroid cancer and ABO blood types and the Rh factor [14]. Blood types have been investigated in the etiology of many malignancies. Metaanalyses have detected a decreased risk of gastric, pancreatic, breast, ovarian, colorectal, esophageal and nasopharyngeal cancers for patients with O blood type. Although associations between some malignancies and blood types have been demonstrated, the link between the expression of blood type antigens and tumor formation has not been clarified for most tumor types in various studies. It was thought that genome-wide association studies may provide prognostic support for the association between the ABO glycosyltransferase gene and cancer risk [13]. In a study, blood group A was associated with significantly higher risk for malignancy including hepatocellular carcinoma, pancreatic and breast cancers while biliary and esophageal cancer risk was significantly associated with blood type B [16]. In another study both B and AB blood types have been associated with a significantly lower risk of gastrointestinal cancer, colorectal cancer. Blood group B was also associated with a significantly lower risk of stomach cancer and bladder cancer, while blood group AB was observed to significantly increase

the risk of liver cancer. It has been found that the risk of gastric cancer, colorectal cancer increases with blood group A [17]. We investigated the relationship between PTC and blood types in the present study.

The distribution of ABO blood types and Rh factor varies between populations and races [18]. In a research conducted in Diyarbakir in Turkey, ABO and Rh blood type ratios were found to be similar to our study. The blood groups of a total of 206.673 people who applied to blood centers were found respectively; 36.55% A Rh(+), 29.70% O Rh(+), 16.65% B Rh(+), 6.26% AB Rh(+), 4.26% A Rh(–), 3.95% O Rh(–), 1.88% B Rh(–), 0.72% AB Rh(–).89.17% of the applicants were Rh positive and 10.82% were Rh negative. As a result, it was stated that there were few differences with the blood group distributions in other regions of our country, and the blood group distribution determined in the study was similar to our country in general [19]. In the present study, the A blood type was the most common blood type, with a slightly higher occurrence rate than the O blood type, which was found at a similar rate to the control group. In the PTC group, blood type A was observed less frequently, whereas blood type O was observed relatively more frequently. The lower occurrence rate of A blood type was statistically significant; however, the higher occurrence rate of O blood type was not statistically significant.

Recent studies have reported a relationship between polymorphisms in the ABO gene locus and circulating tumor necrosis factor-alpha, intercellular adhesion mole- cule-1, E-selectin, and P-selectin levels. It is considered that altered ABO glycosyltransferase activity affects cell proliferation, tumor invasion, and metastatic invasion, thereby playing a key role in carcinogenesis. This suggest- ed that ABO blood type may directly influence tumorigenesis and tumor spread and provide a biological basis for its putative effect on cancer survival. The results of studies evaluating the prognostic significance of ABO antigens in various cancers are rather contradictory. Blood type O appears to be protective against cancer development and progression in pancreatic cancer, whereas the expression of A blood type antigen in tumor cells is reportedly a positive prognostic factor in lung cancer [12]. In our study, A Rh positive blood type was found to be a positive protective factor for PTC.

There are limited data in the literature regarding the relationship between blood types and Rh factor in thyroid cancer. Initially, the keratan sulfate epitope was considered as a specific marker of PTC cells and was observed to be produced simultaneously with poly-N-acetyllac- tosamine, which carries blood type antigens [20]. In another study, the expression of blood type-related antigens was demonstrated in thyroid follicular epithelial cells [21]. In a similar study, it was reported that the neoplastic trans-formation of thyroid gland was accompanied by progressive re-expression of blood type-related antigens, which are not found in normal tissues. The results of that study suggested that poly-N-acetyllactosamine structures are produced in papillary carcinomas in a linear fashion, and these structures are of promising diagnostic value for distinguishing PTC from other thyroid malignancies [22]. Because of these findings that support the role of blood type in cancer

pathogenesis, it is believed that there may be a relationship particularly between PTC and blood type.

In a study investigating the relationship between thyroid cancer, diabetes, and ABO blood type [23], 87 patients with thyroid cancer (68 of them had PTC) were compared with a control group. The most common blood type was O in both groups. It was observed that, compared with the control group, blood type A was significantly less common in patients with thyroid cancer and reduced the risk of thyroid cancer by 43%. Borderline (increased risk of 60%) thyroid cancer risk was reported in patients with blood type B. It was considered that blood type A could significantly reduce the risk of thyroid cancer, whereas blood type B could increase this risk. However, there was no significant relationship between PTC and ABO blood type. The low number of patients with PTC in that study may explain the inability to reveal any relationship with blood type A. As a matter of fact, we found that the risk of PTC decreased significantly by 31% in patients with A Rh positive blood type in our study (OR:0.69, 95% CI: 0.50–0.96). In addition, in the present study, although blood type B was found to be relatively more common in the PTC group, this increase was not statistically significant.

In another study evaluating Rh factor together with ABO blood types [14], 1299 patients with benign and 744 patients with malignant thyroid disease (700 of them had PTC) were compared based on their histopathological features. Individuals with malignant disease were more frequently Rh positive than individuals with benign disease. However, no significant relationship was noted between ABO blood types and thyroid malignancy. Blood type B was considered to potentially contribute to the development of thyroid cancer and as a risk factor for autoimmune thyroid disease. It was considered that the aforementioned study was not sufficient to evaluate the differences in blood types of patients with thyroid cancers and the nor- mal population because it only compared patients with benign thyroid disease and those with malignant thyroid disease. In our study, no significant difference was found between the PTC group and the control group in terms of blood Rh factor.

The limitation of our study in cancer risk assessment was the inability to evaluate the genetic transition of PTC and the environmental exposure of the patients because it was a single-centered, retrospective study.

In conclusion, genetic transitions and potential expo- sure to environmental carcinogens and ionizing radiation are considered to be the main factors responsible for the pathogenesis of thyroid cancer. However, A Rh positive blood type can be considered as a protective factor indicating a reduced risk of occurrence of PTC.

Acknowledgment

We would like to thank Fatma Şener who contributed to the collection of data in our study.

References

[1]Gallardo, E., Medina, J., Sánchez, J.C., Viúdez, A., Grande, E., Porras, I., Ramón, Y., Cajal, T., Trigo, J., Iglesias, L., Capdevila, J., 2020. SEOM clinical guideline thyroid cancer. Clin Transl Oncol. 22(2), 223-35.

[2]Fagin, J.A., Wells, S.A.Jr., 2016. Biologic and Clin- ical Perspectives on Thyroid Cancer. N Engl J Med.

375(11), 1054-67.

[3]Haugen, B.R., Alexander, E.K., Bible, K.C., et al., 2016. American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 26(1), 1-133.

[4]Filetti, S., Durante, C., Hartl, D., Leboulleux, S., Locati, L.D., Newbold, K., Papotti, M.G., Berruti, A., 2019. Thyroid cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Ann Oncol. 30(12), 1856-83.

[5]Cabanillas, M.E., McFadden, D.G., Durante, C., 2016. Thyroid cancer. Lancet. 388(10061), 2783-95.

[6]Pellegriti, G., Frasca, F., Regalbuto, C., Squatrito, S., Vigneri, R., 2013. Worldwide increasing incidence of thyroid cancer: update on epidemiology and risk factors. J Cancer Epidemiol. 965212.

[7]Schneider, D.F., Chen, H., 2013. New developments in the diagnosis and treatment of thyroid cancer. CA Cancer J Clin. 63(6), 374-94.

[8]Ain, K.B., 1995. Papillary thyroid carcinoma. Etiology, assessment, and therapy. Endocrinol Metab Clin North Am. 24(4), 711-60.

[9]Sadeghi, H., Rafei, M., Bahrami, M., Haghdoost, A., Shabani, Y., 2018. Attributable risk fraction of four lifestyle risk factors of thyroid cancer: a meta-analy- sis. J Public Health (Oxf). 40(2), e91-e98.

[10]Seib, C.D., Sosa, J.A., 2019. Evolving Understanding of the Epidemiology of Thyroid Cancer. Endocrinol Metab Clin North Am. 48(1), 23-35.

[11]Dzieczkowski, J.F., Anderson, K.C., 2011. Transfusion Biology and Therapy. Harrison's Principles of Internal Medicine. Longo DL, Kasper DL, Jameson JL, Fauci AS, Hauser SL, Loscalzo J (eds). 18th ed. New York. McGraw-Hill Education. (1), 951-7.

[12]Franchini, M., Liumbruno, G.M., Lippi, G., 2016. The prognostic value of ABO blood group in cancer patients. Blood Transfus. 14(5), 434-40.

[13]Rummel, S.K., Ellsworth, R.E., 2016. The role of the histoblood ABO group in cancer. Future Sci OA. 2(2), FSO107.

[14]Tam, A.A., Özdemir, D., Fakı, S., Bilginer, M.C., Ersoy, R., Çakır, B., 2020. ABO Blood Groups, Rh Factor, and Thyroid Cancer Risk: To 'B' or Not to 'B'.

Endocr Res. 45(2), 137-46.

[15]Nix, P., Nicolaides, A., Coatesworth, A.P., 2005. Thy- roid cancer review 2: management of differentiated thyroid cancers. Int J Clin Pract. 59(12), 1459-63.

[16]Mansour, A., Mohammed, M.A., Anwar, R., Elzafrany, M., Omar, N.M., 2014. ABO blood group and risk of malignancies in Egyptians. Int J Cancer Res. 10(2), 81-95.

[17]Huang, J.Y., Wang, R., Gao, Y.T., Yuan, J.M., 2017.

ABO blood type and the risk of cancer - Findings from the Shanghai Cohort Study. PLoS One. 12(9), e0184295.

[18]Kumar, S., Modak, P.K., Ali, S.H., Barpanda, S.K., Gusain, V.S., Roy, R., 2018. A retrospective study: ABO and Rh phenotype blood group distribution among blood donors in H.N.B. Base Hospital, Sri- nagar, Uttarakhand, India. J Family Med Prim Care. 7(1), 34-8.

[19]Temiz, H., Altıntaş, A., Gül, K., 2008. Distribution of ABO and Rh Blood Groups in Diyarbakir. Interna- tional Journal of Hematology and Oncology. 4(18), 234-7.

[20]Ito, N., Yokota, M., Nagaike, C., Morimura, Y., Ha- take, K., Tanaka, O., Matsunaga, T., 1996. Simulta- neous expression of keratan sulphate epitope (a sul- phated poly-N-acetyllactosamine) and blood group ABH antigens in papillary carcinomas of the human thyroid gland. Histochem J. 28(9), 613-23.

[21]González-Cámpora, R., García-Sanatana, J.A., Jordà i Heras, M.M., Salaverri, C.O., Vázquez-Ramírez, F.J., Argueta-Manzano, O.E., Galera-Davidson, H., 1998. Blood group antigens in differentiated thyroid neoplasms. Arch Pathol Lab Med. 122(11), 957-65.

[22]Yokota, M., Ito, N., Hirota, T., Yane, K., Tanaka, O., Miyahara, H., Matsunaga, T., 1995. Histochemical differences of the lectin affinities of backbone poly- lactosamine structures carrying the ABO blood group antigens in papillary carcinoma and other types of thyroid neoplasm. Histochem J. 27(2), 139-47.

[23]Khoshsirat, S., Peyvandi, A.A., Oroei, M., Peyvandi, H., Rezaeifard, A., 2019. The Estimation of Thyroid Cancer Risk based on Type 2 Diabetes Mellitus and ABO Blood Group. Turk J Oncol. 35(1), 64-9.

Study of the Antitumor Activity of the Drug Dekoglitz on Two Tumors and Some Aspects of Its Mechanism of Action

Zulfiya M. Enikeeva1* Adil A. Ibragimov1* Nigora A. Agzamova1 Natalia L. Vypova1 Saida S. Saidhodjaeva1 Noroj R. Kholturaeva1 Arzayim Ch. Abdirova1 Otabek D. Tuy- chiev1 Jamilya Sh. Polatova1 Dilbar A. Kadirova2 Faizullo S. Salihov3

1.Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology, 700174, Tashkent, Street

Farobi 383, Uzbekistan

2.Institute of Biophysics and Biochemistry at the National University of Uzbekistan, 700174-Tashkent, Street Talabalar 174, Uzbekistan

3.The Bukhara Oncological Centre. Bukhara, Street 200171-Gijduvan Uzbekistan

ABSTRACT

Aim: Evaluation of the antitumor activity of the new drug Dekoglitz in animals with tumor strains of Sarcoma 45 in comparison with the drug dekocin, from which it was obtained, as well as with 5-fluorouracil and etoposide, and on ovarian tumors (OT) in comparison with the drug dekocin and identification of the effect of Dekoglitz on NA synthesis and internucleosomal DNA degradation. Methods: The study of preparations was carried out on 68 outbred rats with transplanted C-45 and OT tumors. The alkylating effect of the drugs was studied on cells tumor of Sarcoma 180. Results: The antitumor activity of dekoglitz on Sarcoma 45 was high, about 98/96%, with a remission rate of 80%. Its effect was 28-24% higher than that of dekocin. On OT, the effect of decoglitz with intraperitoneal administration reached 89/76% with a remission rate of 40%, with oral administration 96/86% with a remission rate of 60%. Conclusion: The study of the new drug Dekoglitz on animals with a tumor of Sarcoma 45 revealed its higher activity (by 20-27%) in comparison with the original Dekocin, 5-fluorouracil and etoposide with a lower level of side effects. On OT, the effect of Dekoglitz was 35-40% higher, especially after oral administration. Apparently, the great ability to suppress the synthesis of NA and carry out internucleosomal degradation and fragmentation of tumor DNA by the new drugs dekoglitz explains its antitumor efficacy,which is greater than that of Dekocin (K-18) in experiments on tumors.

Keywords: Dekocin;Decoglitz;Animal tumors;DNA/RNA

1.Introduction

The pronounced general toxic effect of a large number of used cytostatics, rapidly developing resistance, and the lack of sensitivity of a number of tumors to existing drugs dictate the need to create new anticancer drugs.

The use of medicinal preparations based on licorice root has been around for several

millennia. The main active ingredient in licorice root is the triterpenoid glycyr- rhizic acid (GA). GA as a solubilizer of many water-in- soluble organic substances is used to create low-dose, low-toxic drugs [1].

For example, practically insoluble in water, gossypol and its derivatives, hydrocortisone, prednisolone, kra-cil, nistatine and other drugs in combination with the monoammonium salt of glycyrrhizic acid (MASGA) pass into aqueous solutions [2,3]. All these positive properties of GA and its derivatives are associated with its ability to form supramolecular complexes, which in aqueous solutions have very low critical micelle concentration values. All researchers note the very low toxicity of preparations with GA, MASGK and their derivatives, created on their basis. In addition to the above properties, GA and its derivatives exhibit a pronounced anti-inflammatory, analgesic effect, anti-edema, hypotensive, virus-neutralizing effect, improves tissue regeneration both in the early manifestations of a viral disease and in ulcerative forms.

However, GA was not used for combination with anticancer drugs. We are developing new promising sub- stances based on tropolone alkaloidиs, of which dekocin, a derivative of the alkaloid colchicine, revealed activity in animal studies with 10 tumor strains, which was the highest (above 80%) on Sarcoma S 180, RShM-5 (cervical cancer) and AKATOL [4], which allowed this drug to be proposed for clinical trials. The obtained clinical data of the antitumor drug dekocin indicate a high sensitivity of skin cancer to 3-4% dekocin ointment, which was also effective in combination with radiation [5,6]. However, dekocin is insoluble in water, which complicates both its parenteral administration and bioavailability. In this regard, we used the method of molecular encapsulation of the drug dekocin with glycyrrhizic acid (HA), which has effective solubilizing properties. A new water-soluble supramolecular complex of Dekocin and HA was obtained, which differs in physicochemical parameters from the original Dekocin, as well as a 2.6-fold decrease in toxicity, which is named Dekoglitz.

The aim of this work was to study the antitumor activ-ity of a new colchicine derivative Dekoglitz in animals with tumor strains of Sarcoma 45 and ovarian tumor (OT) in comparison with the effect of decocin, 5-fluorouracil and etoposide, as well as to study the effect of Decoglitz on DNA/RNA synthesis and internucleosomal tumor degradation in comparison with the effect of dekocin (K-18) and etoposide.

2.Methods

2.1Tumoral Strains

Transplantable tumors, murine sarcoma 180, and two strains of rat sarcoma 45 and ovarian tumors (OT) were used in the work. Strain Sarcoma 180 was purchased from the Tumor Strains Collection Bank (Institute of Carcino- genesis, N.N. Blokhin Russian Cancer Research Center, Russian Academy of Medical Sciences) Moscow, Russia. The strains of Sarcoma 45 and ovarian tumors (OT) were purchased from the Tumor Strains Collection Bank (Institute of Oncology of Kazakhstan). The tumor strains were passaged to the strain protocol.

2.2Antitumor Drugs

The following drugs were used in the work: etoposide (Etoposide phosphate, Bristol-Myers Squibb); 5-fluoro- uracil (Getwell Pharm acutikals, India); the K-18 (Dekocin) and its derivative Dekoglitz (tropolone alkaloids, col chicine derivatives) developed by Prof., Z. M. Enikeeva at the Republican Specialized Scientific Practical Medical Center of Oncology and Radiology of the Ministry of Health of the Republic of Uzbekistan (RSNPMTSO&R MH RUz).

2.3Animals

In the experiment white outbred, mice weighing 18-20 g (60 individuals) and rats weighing 90-140 g (68 individuals) were used. The animals were kept on a standard diet under natural lighting conditions and had free access to water and food in the vivarium at the RSNPMTSO&R MH RUz.

At the end of the experiment, all rats and mice were euthanized under ether anesthesia, in accordance with the International Rules for the Protection of Vertebrates. All experiments were performed in accordance with the recommendations and requirements of the "World Society for the Protection of Animals (WSPA)" and "European Convention for the Protection of Experimental" (Strasbourg, 1986).

2.3.1Investigation Antitumor Activity of Drugs

Tumor subinoculation was carried out according to generally accepted methods: tumors of Sarcoma 45 and OT were inoculated subcutaneously with a suspension of tumor cells, 30-60 mg in 0.3-0.5 ml of nutrient medium per rat[7]. Treatment of animals began 4 days after tumor implantation, drugs were injected in all groups 10 times, and all experimental groups were injected with drugs in a volume of 0.3 ml per 100 g rat. The animals were slaughtered on the 19-21st day after tumor implantation, the animals were sacrificed using humane methods of working with laboratory animals. Before the introduction and at the end of the experiment, the body weight of the animals was determined.

During the experiment, in order to study the dynamics of tumor growth, the volumes of tumors through the skin of animals were measured in the treated and control groups of mice (in 3 projections) at the beginning of the experiment, every 5 days after the start of treatment, and before slaughter. At the end of the experiment, the efficacy in sacrificed mice was determined by the volume (V) of the extracted tumor tissue, as well as by the tumor mass in the compared groups. Tumor growth inhibition was calculated using the formulas [7]. The tolerability of the treatment was judged by the death of the mice; for an indirect assessment of the possible hematotoxicity in the sacrificed mice, the spleen weight was determined.

2.3.2Alkylating Action

The effect of drugs on the synthesis of DNA and RNA was studied on Sarcoma 180 tumor cells in vitro. A cell suspension from tumor tissue was obtained according to the method [8]. Cells with a titer of 10,000 were cultured in medium (RPMI-1640 containing 5% fetal bovine serum (FBS), 2mM L-glutamine, 10U/ml penicillin and 100mcg/ ml streptomycin), with the absence and presence of therapeutic (TD) investigational drugs , for 24 hours at 370C in an atmosphere of 5% CO2.

2.3.3Isolation of DNA/RNA

DNA/RNA preparations from Sarcoma 180 cells were isolated by two methods, a) phenol-chloroform method [9] and according to the protocol of the kit-kit "DNA-sorb-B" (InterLabService), Russia. The DNA/RNA concentration was determined by adsorption at a wavelength of 260 nm on an SF-26 spectrophotometer (Russia).

For the analysis of internucleosomal DNA degradation, total DNA/RNA preparations were treated with the RNase A enzyme according by method [9]. DNA/RNA electrophoresis was analyzed in 1.5% agarose gel for 4 h, 60V according to the method [9].

Statistical processing was performed using Statistica, version 6.0. The level of statistical significance was taken as $p < 0.05$.

3.Results

3.1Study of Antitumor Activity, in vivo

The study of the antitumor activity of the drugs on the Sarcoma 45 strain began 4 days after tumor subinoculation the drugs were injected 10 times. The slaughter was carried out on day 21. In the control group, there was a mortality of 25%, in the experimental groups with the use of dekoglits and dekocin, there was no death of animals, in the group with 5-fluorouracil all animals died after 10- fold administration, in the group with etoposide, 30% of the animals died.

In group 2, the drug Dekoglitz showed high antitumor activity in 98/96%, 80% of regressed tumors were ob- served, while the drug caused a slight decrease in body weight (by 5%) and an increase in the spleen by 20% (Ta- ble 1).

In group 3, the antitumor effect of the drug dekocin was less high - 70/72%, the drug caused a slight decrease in body weight (by 6%) and spleen (by 20%).

In group 4, the drug 5-fluorouracil at a dose of 15 mg/ kg caused the death of all animals on day 15 after inoculation and its antitumor effect could be assessed on day 12 when measuring the volume of tumors in 2 animals, 12 when measuring the volume of tumors in 2 animals, which was in relation to the control for this day is 76%, however, due to the death of animals, it was impossible to assess the effect of the drug on body weight and spleen.

Table 1. Antitumor activity of the drug Dekoglitz in comparison with Dekocin, 5-FU and Etoposide in rats with tumor Sarcoma 45(Treatment with drugs was carried out on the 4th day after tumor implantation. The slaughter was carried out on the 21st day)

Groups of animals	Number of animals before and after treatment		The mass of animals (gr)		Tumor volume, (cm^3)		
	before	after	before	after	5th day	12th day	after
Control	8	6	131.0±9.3	121.3±9.0	0.3±0.04	2.1±0.5	2.7±0.8
Decoglitz 20 mg/kg	6	6	102.0±5.8	97.0±6.0	0.2±0.1	0.2±0.1	0.04±0.01*
Dekocin 15 mg/kg	6	6	132.0±17	124.0±14.4	0.1±0.02	0.5±0.13	0.8±0.07*
5-FU 15 mg/kg	6	0	109.0±4.4	-	0.1±0.05	0.48±0.08	-

Note: in the treatment groups n = 6, in the control n = 8; * differences are statistically significant in comparison with control at $P < 0.05$.

Table 1. Continued.

Groups of animals	Weight tumors (gr)	Weight Spleen (mg)	by volume	% inhibition of tumor by mass	growth % regression
Control	2.5±1.1	0.5±0.03			
Decoglitz	0.04±0.01	0.6±0.04	98	96	80
Dekocin	0.7±0.07*	0.4±0.03	70	72	0
5-FU	-	-	77	-	0
Etoposide	0.6±0.06*	0.3±0.02	78	76	0

Table 2. Antitumor activity of the drug Dekoglitz in comparison with Dekocin in rats with ovarian tumor(Treatment with drugs was carried out on the 4th day after tumor implantation. 10 injections of substances. The slaughter was carried out on the 19th day)

Groups of animals	Weight of animals (gr)		Tumor volume (cm^3)		Weight tumors (gr)	Weight spleen (mg)	% inhibition of tumor growth		
	before treatment	after treatment	for 5th day	for 10th day			by volume	by mass	% regression
Control	160±10.6	162±10.2	1.7±0.5	2.8±0.5	2.5±0,6	0.9±0.07			
Dekocin, 15 g/kg	97±1.3	118±0.02	0.2±0.02	1.3±0.1*	1.4±0.4*	0.8± 0.1	54	44	
Decoglitz 20 mg/kg, (intra-peritoneal)	119±4.6	135±7.0	0.7±0.1	0.3± 0.02*	0.6±0.1*	0.8±0.07	89	76	40
Decoglitz 40 mg/kg, (orally)	110±3.2	139±5.7	0.6±0.1	0.1±0.01*	0.1±0..03*	0.8±0.1	96	86	60

Note: in the treatment groups n = 6, in the control n = 6; * differences are statistically significant in comparison with control at P <0.05.

In the 5th group, the antitumor effect of the drug etoposide was 78/76%, the drug caused a slight decrease in body weight (by 7%) and a more pronounced decrease in the spleen weight (by 40%).

Thus, the new drug Dekoglitz showed the highest activity, both in comparison with dekocin, from which it was obtained, and known cytostatics, moreover, its effect was higher than the comparison drugs by 20-28%, and there was no such side effect as the effect on the spleen. It should be noted that Dekoglitz was studied at a dose that in relation to LD50 was significantly lower than that of de kocin, i.e. for GA and MASGK derivatives, it was noted [10] that their activity manifests itself in doses 2-4 times less than the maximum tolerated.

The study of the antitumor activity of the drugs on the Ovarian Tumor (OT) strain began 4 days after tumor transplantation the drugs were injected 10 times. There was no death of animals during the experiment. The slaughter was carried out on the 19th day.

In group 2, the drug dekocin was 54/44% active, while the drug caused a slight decrease in body spleen weight (by 11%) and an increase in body weight by 21% (Table 2).

In group 3, the antitumor effect of the

drug Dekoglitz at a dose of 20 mg / kg with intraperitoneal injection was less high - 89/76% than when exposed to Sarcoma 45, but caused tumor regression in 40% of animals. The drug caused a slight decrease in the spleen (by 11%), body weight was 13% more than the initial one.

In the 4th group, the drug Dekoglitz at a dose of 40 mg/ kg with oral administration had a higher antitumor effect 96/86%, while it caused tumor regression in 60% of animals, the drug had side effects only in a slight decrease in the spleen (by 11%), body weight was 26% more than the initial one.

3.2Study of the Mechanism of Action, in vitro

The high antitumor activity of the drug Dekoglitz, as well as its further study as a cytostatic, involves the study of such aspects of its mechanism of action as alkylating, the influence on DNA/RNA synthesis, internucleosomal DNA degradation, and topoisomerase II activity. The ef- fect of K-18 and Decoglitz on DNA and RNA synthesis was investigated in sarcoma 180 cells in vitro in com- parison with etoposide, which is a known inhibitor of to- poisomerase I/II.

Figure 1 shows the results of DNA/RNA electrophoresis of tumor cells cultured in the absence of preparations (lanes 1,2) and using etoposide, K-18 and Dekoglitz (respectively, lanes 4-6).

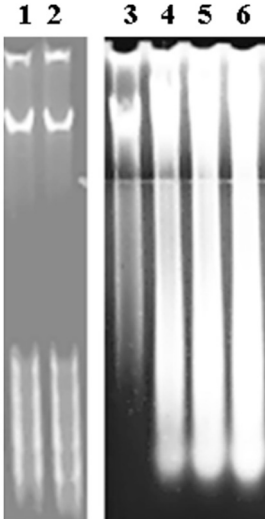


Figure 1. Influence of preparations on native DNA, nucle- osoma degradation of DNA and activity topoisomerase II tumor cells of the Sarcoma 180, in vitro

Lanes 1 and 2 native DNA/ RNA of Sarcoma 180 (not treated with RNase A). Lanes 3-4, aliquots of DNA treat- ed with RNase. Lane 3 control, without the using of cyto- statics, Lane 4 Etoposide, Lane 5 K-18 (Dekocin), Lane 6 Dekoglitz. Electrophoresis carries out in to 1.5 % TAE agarose gel, 4h, and 60V and visualized by UV transillu- minator after staining with ethidium bromide.

In aliquots of DNA/RNA not treated with the enzyme RNase, the electrophoregram shows a high native of nu- clear DNA and RNA molecules (Figure 1, lanes 1, 2). In aliquots of DNA/RNA treated with the enzyme RNase, the electrophoregram shows DNA degradation in the form of a plume (Figure 1, lanes 4-6).

The results of the electrophoresis showed: Etoposide, K-18, and Dekoglitz contributed to internucleosomal DNA degradation by: 75.7 ± 3.3, 86.7 ± 3.7, and 94.5 ± 1.7, respectively. Also, according to the pattern of DNA

fragmentation, electrophoregram, Etoposide, K-18, and Dekoglitz inhibited topoisomerase II activity by 57.6 ± 2.7,

64.6 ± 2.3, and 79.6 ± 3.0, respectively (Figure 1, Table 3). Regarding the effect of the three drugs on topoisomerase II (TOPO-II) of Sarcoma 180 tumor cells, the activity of this enzyme was determined visually by the pattern of electrophoresis of fragmented DNA in a gel. Etoposide, K-18, and Dekoglitz inhibited TOPO II activity by 57.6± 2.7, 64.6 ± 2.3, and 79.6 ± 3.0, respectively (Figure 1,Table 3).

The results, the effect of the studied drugs on the syn- thesis of DNA/RNA showed: a) Etoposide, K-18 and Dekoglitz inhibited DNA synthesis by 64.9 ± 2.7, 85.6 ± 2.3, 95.7 ± 3.7, respectively; b) Etoposide, K-18, and Dekoglitz inhibited RNA synthesis by 30.0 ± 3.0, 60.5 ± 1.7,

65.9 ± 2.7, respectively (Table 3).

Table 3. Influence of antineoplastic preparations on syn- thesis DNA/RNA, TOPO II activity and DNA nucleosoma degradation of cells of the Sarcoma 180 tumor, in vitro

Antitumor preparations (TD)	DNA nucleosoma degradation, in %	Inhibition		
		Activity TOPO-II in %	DNA synthe-sis in %	RNA synthe-sis in %
Control	0±0	0±0	0±0	0±0
Etoposide 8 mkg/ml	75.7±3.3	57.6±2.7	64.9±2.7	30.0±3.0
K-18 15 mkg/ml	86.7±3.7	64.6±2.3	85.6±2.3	60.5±1.7
Decoglitz 20 mkg/ml	94.5±1.7	79.6±3.0	95.7±3.7	65.9±2.7

4.Conclusion

The study of the new drug Dekoglitz on animals with a tumor of Sarcoma 45 revealed a very high activity with 80% tumor regression, which was 20-27% more than the original Dekocin, 5-fluorouracil and etoposide with a low- er level of side effects. Dekoglitz also had a high effect on OT tumor when administered intraperitoneal, which was 30-40% higher than the effect of Dekocin (40% of tumors regressed), however, Dekoglitz showed an even higher activity after oral administration, where 60% of tumors regressed.

This Dekoglitz effect is confirmed by a more intense effect on the synthesis of DNA and RNA of tumor cells. Apparently, the great ability to suppress the synthesis of NA and the activity of topoisomerase II and to carry out in-ternucleosomal degradation of tumor DNA by the new drug Dekoglitz explains its antitumor

efficacy, which is greater than that of Dekocin (K-18) in experiments on tumors.

Acknowledgement

This work is executed at financial support of fund of applied researches of Republic Uzbekistan (the project № FV 2020196206).

Conflict of Interest

The authors declare no conflict of interest.

References

[1]Tolstikov. G.A., Baltika L.A., Shultz E.E., Pokrovsky A.G. Glycyrrhizic acid in Russian. J. Bioorganic Chemistry, 1997, 23(9): 691-709.

[2]Dalimov D.N., Gafurov M.B., Akhunov A.A., Matchanov A.D., Vypova N.L., Khamidova G.R., Nuritdinova N.S., Toshpulatov F., Esonov R. Supra-molecular complexes of glycyrrhizic acid (structure, biological activity, application) in Russian. Scientific and practical conference "Uzbekistonda tabiy birik- malar kimyosining rivozhi va kelazhagi". Tashkent, 2016: 15-16.

[3]Dalimov D.N., Yuldashev H.A., Gafurov M.B., Vy- pova N.L., Inogamov U.K. Synthesis, structure and study of biological activity of supramolecular com-plexes of glycyrrhizic acid in Russian. Halgaro ylmi- um anzhuman, Tashkent, 2013: 17-18.

[4]Enikeeva Z.M., Ibragimov A.A. New class cytostat- ics with stimulation colony-forming units on a spleen (CFCs) in Russian. Tashkent, J. Fan va texnologiya, 2016: 173.

[5]Holikov T.K., Gafur-Akhunov M.A., Goyibova S., Enikeeva Z.M. New antitumor preparation decocin for the treatment of skin cancer, and igreasing effect of irradiation to the tumor as well. Journal Avicenna- Freiburg - Germany, Heft 2, 2011: 32-37.

[6]Usmanov R.Kh., Rakhmatullaeva D.T., Enikeeva Z.M. Development of schemes for the use of a new domestic antitumor drug dekocin in the treatment of skin cancer of the eyelids and periorbital region in Russian. J. Med. Uzbekistan, 2011(6): 37-39.

[7]Guidelines for studying the antitumor activity of pharmacological substances. Compiled by E.M. Treschalin, O.S. Zhukova, G.K. Gerasimova, N.V. Andronova, A.M. Garin in the book. Guidelines for experimental (preclinical) study of new pharmaco- logical substances in Russian. Ed. R.U. Khabrieva. Moscow, 2005: 637-682.

[8]Samusenko A.V. Mechanisms of cell death under the action of olivomycin and its derivatives in Russian. Author's abstract, Ph.D., Moscow-2009: 19.

[9]Maniatis T., Fritsch E. E., Sambrook J. In the Book: Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, 192: 480.

[10]Enikeeva Z.M., Kholturaeva N.R., Agzamova N.A. New complexes of GK and MASGK with colchicine derivatives in Russian. Thesis of the conference "Medicines based on natural compounds" Tashkent, 2018: 193

Treatment Outcomes of Germ Cell Tumors of Ovary: Single Institutional Study

Rohit Kumar Jha1 Ajit Kumar Kushwaha1 Mukunda Kuma2 Sunaina Wadhwa3 Sumedha Gargy1*

1.Department of surgical oncology, Rajendra Institute of Medical Sciences, Ranchi
2.Department of Biochemistry, All India Institute of Medical Sciences, Patna
3.Ex-fellow, Department of Gynaecological Oncology, TMC, Kolkata

ABSTRACT

5% of all ovarian tumours are accounted to germ cell tumours (GCT's). Affecting mostly young women, the highest incidence is seen in second and third decade of life. They are highly malignant but chemosensitive and more curable than their epithelial counterparts. Treating these tumors with effective surgery and combination chemotherapy survival rates have dramatically improved in recent decades. We present our experience of ovarian germ cell tumours in the department of Surgical Oncology, Rajendra Institute of Medical Sciences (RIMS), Ranchi with special emphasis on treatment outcomes. A retrospective review of hospital medical records of patients with ovarian germ cell tumours diagnosed and treated at RIMS from June 2019 to August 2020, was performed. Clinical profile and treatment outcome of patients were recorded. A total of 19 patients met criteria. The median age at diagnosis was 20 years (range 11- 42 years) and all had good performance status. All except two patients underwent surgery, 70.6% and 29.4% in upfront and interval debulking surgery (IDS) setting respectively. Fertility preserving surgery was done in 75% patients in the primary surgery group and 60% undergoing IDS. 83.3% patients received BEP as adjuvant chemotherapy whereas 80% as neo-adjuvant chemotherapy.Majority (31.5%) patients had dysgerminoma as final histtology, followed by mixed histology(26.3%), yolk sac tumour (15.7%), immature teratoma (15.7%) and choriocarcinoma (10.5%). 47.3% patients were in Stage I at the time of diagnosis. 78.9% patients were alive without disease, 10.5% recurred, and 10.5% were lost to follow up.

Keywords: Germ cell tumour;Dysgerminoma;BEP;Fertility sparing surgery;Yolk sac tumour

1.Introduction

20 to 25% of all benign and malignant ovarian neoplasms are of germ cell origin. These are uncommon neoplasms arising from primitive germ cells of the embryonic gonad. Affecting mostly young women, the highest incidence is seen in second and third decade of life. Most common histology is teratoma followed by dysgerminoma worldwide.

Before mid 1960's, almost all non-dygerminomatous GCT patients died. The patients with dysgerminoma survived owning to its high radiosensitivity but fertility was not spared. The introduction of combination chemotherapy VAC (vincristine, dactinomycin,

and cyclophosphamide) achieved 85% cure rate in stage I GCT's whereas metastatic disease had a 50-70% mortality. Moreover as BEP therapy does not effect ovarian function much, most GCT patients remain fertile and are able to give birth. FIGO stage and elevated tumour markers are seen to be independent poor prognostic indicators

2.Materials and Methods

This is a retrospective study in which data was collected prospectively from hospital medical records. All patients with histologically confirmed GCT and treated at RIMS from June 2019 to August 2020 were included. A total number of 19 patients met criteria.

Clinical profile, stage of presentation, histological classification and treatment received (primary surgery followed by chemotherapy or neo-adjuvant chemotherapy followed by interval debulking surgery) was seen. Treatment outcome and disease free interval were ascertained.

The data was analysed using SPSS software.

3.Results

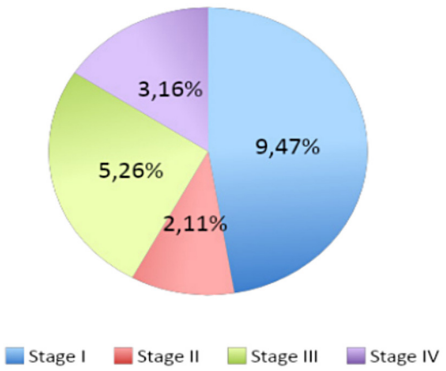
During this 14 month period, a total of 19 patients were admitted and treated in our department. The median age was 20 years (range 11-42yrs) out of which one patient was premenarchal. All patients had a performance status of ECOG 0/1. The median AFP at presentation was 986 ng/mL (range 11-23000 ng/mL), median LDH 843 U/L (range 273-15000 U/L). All except two patients had normal β-hCG (table 1).

Stage wise distribution is depicted in Figure 1. Majority patients(n=9; 47.3%) belonged to stage I followed by stage III (n= 11; 26.3%). Two patients belonged to stage II and three to stage IV.

Table 1. Demographics

Median Age	20 (11-42years)
PS	All except 1 were ECOG 0/1
Tumour markers at presentation	
AFP	986 (11-23,000)
LDH	843 (273-15000)
bHCG	Normal in all except 2

Figure 1:FIGO Stage Distribution



12 (70.6%) patients underwent upfront surgery out of which 9 (75%) had fertility preservation and 3 (25%) had radical surgery.

In terms of adjuvant chemotherapy, 10 (83.3%) patients received BEP to begin with following which 3 (30%) were switched to EP due to bleomycin induced toxicity, 2 (16.6%) patients received EP, whereas 1 (8.3%) received VIP after disease progression on BEP.

Table 3. Interval debulking surgery details

Surgical details: Upfront surgery		
Type	Details	N=12
Fertility preserved	USO	3
	BSO	1
	USO/omentum	1
	USO/nodes/omentum	4
Fertility not pre-served	TAH BSO	1
	TAH BSO/nodes/omentum	2

Table 2. Upfront surgery details

Surgical details: Interval debulking surgery		
Type	**Details**	**N=5**
Fertility preservation	USO/omental biopsy	2
	USO/omentectomy	1
Fertility not preserved	TAH BSO/omentectomy/ LND	2

7 patients received neo-adjuvant chemotherapy (4 BEP followed by VIP in 1 patient, 1 EP and 2 single agent Methotrexate) following which 5 underwent interval debulking surgery (2 patients had choriocarcinoma which was cured with chemotherapy). The reasons for NACT were extensive disease in 2 patients out of which 1 patient also had portal vein thrombosis, poor PS in 2 patients, whereas 1 patient had already received NACT before she presented to us. Among the 5 patients undergoing interval debulking surgery 3 had fertility preservation. Complete cytoreduction of macroscopic disease was achieved in all patients who underwent surgery.

Among the 10 patients receiving adjuvant BEP chemotherapy following upfront surgery, majority received 4 cycles (6 patients) out of which 2 patients received subsequent 2 cycles of EP, 3 patients received 3 cycles out of which 1 received subsequent 2 cycles of EP and 1 received additional 3 cycles of VIP. 1 patient was switched to 3 cycles of EP regimen after the first cycle of BEP due to bleomycin toxicity and 2 patients received 4 cycles of EP.

In the interval debulking surgery group, 3 patients received 3 cycles of BEP, 1 received 4 cycles BEP whereas 1 received 1st cycle as single agent carboplatin followed by 4 cycles EP as NACT. The patients with choriocar-cinoma received 4 cycles of single agent methotrexate. Post-operatively 2 patients received 2 cycles EP whereas the rest did not need adjuvant treatment.

Table 4. Chemotherapy details

Setting	Regimen	*n* = 19
NACT	BEP	3
	EP	2
	Methotrexate	2
	TOTAL	7
Adjuvant	BEP	10
	EP	2
	TOTAL	12

Table 5. Chemotherapy cycles

Setting	Cycles	n = 40
NACT	Three	3
	Four	4
Chemotherapy (After IDS)	Two	2
	Six	1
	Three	2
Adjuvant Chemotherapy	Four	7
(after upfront surgery)	Five	1
	Six	2

19 (47.5%) patients had yolk sac tumour, 11 patients had mixed histology, 5 presented with dysgerminoma, 4 with immature teratoma whereas 1 patient had choriocar-cinoma as depicted in figure 2.

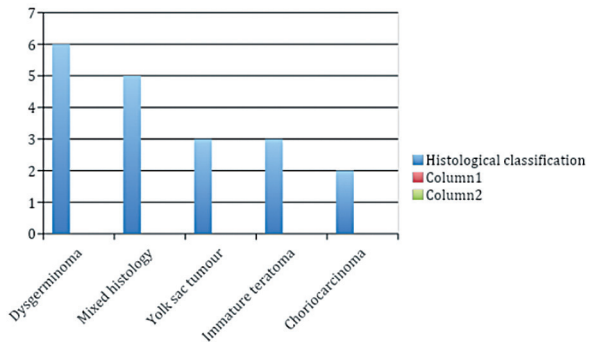


Figure 2.

Follow up data was available for n= 17 patients. This data revealed 15 patients alive without disease and 2 patients alive with disease. 2 patients developed recurrence and 2 were lost to follow up. None of the patients died during this period.

In the recurrence group, one patient had recurrence in both abdomen and pelvis whereas another one in abdomen and thorax. Both received second-line chemotherapy. Two patients were lost to follow up.

4.Discussion

Though accounting for only 5% of all malignant neoplasms, their impact in patient's life is enormous since it mostly affects women in their second or third decade of life. Fearing the sequelae of the disease, many women choose radical surgery but it has been seen that fertility sparing surgery is also an equally effective treatment with possibility of future childbearing. It is now possible to achieve complete cure with fertility preservation however reports on reproductive outcome of GCT survivors are sporadic since it has been only around 30 years since this dramatic improvement due to the implementation of BEP chemotherapy. However Zhang et al. in their retrospective study of 32 patients with malignant ovarian germ cell tumour (MOGCT) and sex cord stromal tumours (SCST) showed fertility sparing surgery to be an equally effective alternative to radical surgery with the advantage of menstrual and fertility preservation [10]. In their series all patients underwent fertility sparing surgery (FSS) whereas we could achieve 70.5% FSS rate. This could be due to the fact that all except one patient in their series belonged to either stage I or II whereas 42.1% patients in our series had stage III-IV disease. In addition to a desire to cure, a desire to become pregnant also improves treatment outcomes.Similarly, Turkmen et al. in their series of 69 patients compared survival outcomes of patients undergoing conservative surgery with definitive surgery and established surgery type to be insignificant for recurrence. Other studies have also shown that fertility sparing surgery did not affect cancer prognosis in cases of advanced germ cell tumours.

Maheshwari A et al. in their report from India,stated dysgerminoma as the most common histological type which is similar to our series of patients.

Though majority of patients belonged to stage I, a considerable number of patients presented in the advanced stage. This is due to proper staging surgeries performed at our centre along with late referral from other non-on-cologic treating institutes. Many patients were operated outside without suspicion of malignancy and presented to us with suspicious final histopathology. One patient with advanced disease even received neo-adjuvant chemotherapy outside before presenting to

us. BEP chemotherapy is effective for treating germ cell tumors of the ovary and it has been validated in several trials.

In the NACT setting, there was complete pathological(or radiological) response in 66.67% patients whereas the rest showed partial response according to the RECIST criteria. S Talukdar et al in their series of 23 patients receiving NACT also showed a complete response of 60.5%. Literature reports 40.7% complete pathological response.10.5% patients recurred and were managed with second line chemotherapy such as VIP (etoposide, ifosfamide, and cisplatin).

Most patients with early disease and healthy looking uterus and ovary underwent fertility sparing surgery. In cases of normal looking contralateral ovary, routine biopsy was not taken however it was thoroughly palpated and any suspicious nodules biopsied. Although occult bilaterality has been reported, biopsy of the contralateral ovary could lead to future infertility related to peritoneal adhesions or ovarian failure. Unlike their epithelial ovarian counterparts, GCT's are amenable to fertility preservation. Hence it should be the standard of care whenever possible. Many studies document good outcome in this regard.

References

[1]Gershenson DM, Copeland LJ, Kavanagh JJ, Cangir A, Junco GD, Saul PB, et al. Treatment of malignant nondysgerminomatous germ cell tumors of the ovary with vincristine, dactinomycin, and cyclophosphamide. Cancer, 1985, 56: 2756-61.

[2]Slayton RE, Park RC, Silverberg SG, Shingleton H, Creasman WT, Blessing JA. Vincristine, dactinomycin, and cyclophosphamide in the treatment of malignant germ cell tumors of the ovary. A Gynecologic Oncology Group Study (a final report). Cancer, 1985,56: 243-8.

[3]Gershenson BDM. Menstrual and reproductive func- tion after treatment with combination chemotherapy for malignant ovarian germ cell tumors. Am Soc Clin Oncol, 2016, 6: 270-5.

[4]Brewer BM, Gershenson DM, Herzog CE, et al. Out- come and reproductive function after chemotherapy for ovarian dysgerminoma. J Clin Oncol., 2017, 17: 2670-5.

[5]Kanazawa K, Suzuki T, Sakumoto K.Treatment of malignant ovarian germ cell tumors with preservation of fertility: reproductive performance after persistent remission. Am J Clin Oncol., 2000, 23: 244-8.

[6]Tangir J, Zelterman D, MaW, et al. Reproductive function after conservative surgery and chemothera- py for malignant germ cell tumors of the ovary. Ob- stet Gynecol., 2003, 101: 251-7.

[7]Murugaesu N, Schmid P, Dancey G, et al. Malignant ovarian germ cell tumors: Identification of novel prognostic markers and long term outcome after multi-modality treatment. J Clin Oncol., 2006, 24: 4862-4866.

[8]Downey J, McKinney M. The psychiatric status of women presenting for infertility evaluation. Am J Orthop, 1992, 62: 196-198.

[9]Zanetta G, Bonazzi C, Cantu M, Binidagger S, Locatelli A, BratinaG, Mangioni C. Survival and reproductive function after treatment of malignant germ cell ovarian tumors. J Clin Oncol., 2001, 19(4): 1015-1020.

[10]Zhang N, Chen R, Hua K, Zhang Y. A retrospective study of reproductive outcomes after fertility-sparing surgery and postoperative adjuvant chemotherapy in malignant ovarian germ cell tumors and sex cord-stromal tumors. Journal of Ovarian Research, 2017, 10: 52.

[11]Turkmen O, Karalok A, Basaran D, Kimyon G. Fertility-Sparing Surgery Should Be the Standard Treatment in Patients with Malignant Ovarian Germ Cell Tumors. Journal of Adolescent and Adult Oncology, 2017. DOI: 10.1089/jayao.2016.0086

[12]Maheshwari A, Gupta S, Parikh PM, Tongaonkar HB. Malignant germ cell tumor ovary-experience at Tata Memorial Hospital. Indian J Med Paediatr On- col., 2004, 25(1): 43.

[13]Gershenson DM, Morris M, Cangir A, Kavanagh JJ, Stringer CA, Edwards CL, Silva EG,Wharton JT. Treatment of malignant germ cell tumors of the ovary

with bleomycin, etoposide and cisplatin. J Clin Oncol., 1990, 8(4): 715-720.

[14]De Wit R, Roberts JT et al. Equivalence of three or four cycles of bleomycin, etoposide and cisplatin combination therapy and of a 3 or 5 day schedule in good prognosis germ cell cancer: a randomized study of the European Organization for Research and Treatment of Cancer Genitourinary Tract Cancer Co- operative Group and the Medical Research Council. J Clin Oncol., 2001, 19(6): 1629-1640.

[15]Saxman SB, Finch D, Gonin R, Einhorn LH. Long term follow up of a phase III study of three versus four cycles of bleomycin, etoposide and cisplatin in favourable prognosis germ cell tumors: the Indian University experience. J Clin Oncol., 1998, 16(2): 702-706.

[16]Talukdar S, Kumar S, Bhatla N , Mathur S, Thulkar S, Kumar L. Neo-adjuvant chemotherapy in the treatment of advanced malignant7germ cell tumors of ovary. Gynecologic Oncology, 2014, 132: 28-32.

[17]Culine S, Lhomme C, Kattan J, Michel G, Duvillard P, Droz JP. Cisplatin based chemotherapy in the management of germ cell tumors of the ovary: the Institut Gustave Roussy Experience. Gynecol Oncol., 1997, 64(1):160-165.

[18]Gershenson DM. Management of early ovarian can- cer: germ cell and sex cord-stromal tumors. Gynecol Oncol., 1994, 55: S62-72.

[19]Weinstein D, Polishuk WZ. The role of wedge resection of the ovary as a cause for mechanical sterility. Surg Gynecol Obstet, 1975, 141: 417-8.

[20]Perrin LC, LowJ, Nicklin JLWardBG, Crandon AJ. Fertility and ovarian function after conservative surgery for germ cell tumors of the ovary. Aust N Z J Obstet Gynecol, 1999, 39(2): 243-245.

[21]Ezzat A, Raja M, Bakri Y, Subhi J, Memon M, Schwartz P, Stuart R. Malignant ovarian germ cell tumors: a survival and prognostic analysis. Acta On- col., 1999, 38(4): 455-460.

[22]Brewer M, Gerhenson DM, Herzog CE Mitchell MF, Silva EG, Wharton JT. Outcomes and reproductive function after chemotherapy for ovarian dysgermino-ma. J Clin Oncol., 1999, 17(9): 2670-2675.

[23]Park J, Kim D, Suh D, Kim J, Kim Y. Outcomes of pediatric and adolescent girls with malignant ovarian germ cell tumors; Gynecol Oncol, 2015. http://dx.doi.org/10.1016/j.ygyno.2015.03.054

[24]Weinberg L, Lurain J, Singh D, Schink J, Survival and reproductive outcomes in women treated for malignant ovarian germ cell tumors. Gynecologic Oncology, 2011, 121: 285-289.

[25]Tamauchi S, Kajiyama H, Yoshihara M, Ikeda Y, Yoshikawa N, Nishino K, Utsumi F, Niimi K, Suzuki S, Kikkawa F. Reproductive outcomes of 105 malignant ovarian germ cell tumor survivors: a multicenter study. Am J Obstet Gynecol., 2018, 219(4): 385.e1- 385.e7.DOI: 10.1016/j.ajog.2018.07.021. Epub 2018 Aug 4.PMID: 30086295

[26]Patterson DM, Murugaesu N, Holden L, et al. A review of the close surveillance policy for stage I female germ cell tumors of the ovary and other sites. Int J Gynecol Cancer, 2008, 18: 43-50.24. Park J Y, Kim D Y, Suh D S,et al. Analysis of out- comes and prognostic factors after fertility-sparing surgery in malignant ovarian germ cell tumors. Gynecol Oncol., 2017, 145: 513-8.

High Grade Muscle-Invasive Urinary Bladder Cancer in A 36 Year Old Male Patient: A Case Report & Review of Literature

Sachin S Kadam1* Rohan Jagtap2 Tejaswini Kadam3
1.Department of Surgical Oncology, Currae Cancer & Multispeciality Hospital, Mumbai, India
2.Department of General & Laparoscopy Surgery, Vedant Cancer & Multispeciality Hospital, Mumbai, India
3.Department of Ophthalmology, Conwest & Jain Superspeciality Eye Hospital, Mumbai, India

ABSTRACT

We are reporting a case of urothelial bladder cancer in a 36 year old male patient with no history or exposure to any risk factors. The incidence of urothelial bladder cancer is very low in young individuals i.e. below 40 years of age with reported rate of incidence around 0.1-0.4 %. Most of the times, these young individuals present with non-muscle invasive bladder cancer with low grade and low stage. As the age increases, the incidence of high grade bladder cancer increases along with it. The index case presented with high grade muscle invasive bladder cancer at the time of diagnosis without any known risk factors. The 5-year survival of urothelial bladder cancer is better in young patients (93.8 %) as compared to older people(85.1 %). Cigarette smoking is responsible for development of bladder cancer in majority of patients followed by exposure to occupational carcinogens. Role of genetic alterations in development of bladder cancer is still under research and process of urothelial bladder carcinogenesis is unanswered in young individuals.

Keywords: Urothelial baldder cancer;High grade;Muscle invasive;Young adult;Risk factors;Prognosis

1.Introduction

Urothelial bladder cancer is a rare presentation in young individuals, preferably below 40 years of age with reported incidence rate of around 0.1% to 0.4% in the first two decades of life and majority of patients are above 60 years of age at the time of diagnosis. Worldwide, bladder cancer ranks at 11th position with incidence 3%, mortality around 2.1% and 5-year prevalence around 22.07%. In India, bladder cancer ranks at 17th position with incidence 1.6%, mortality 1.3% and 5-year prevalence around 3.57%. In developed countries, Squamous cell carcinoma of the bladder is a rare cause of bladder cancer with accountability of 2.7% of all bladder cancer cases. Around 59% of bladder cancer cases are caused by schistosomiasis where it is endemic. The sub- classification of squamous cell carcinoma of the bladder is bilharzial and nonbilharzial which depends upon the causative agent, Schistosomiasis hematobium. About 30% of newly diagnosed bladder cancer patients present with muscle invasive bladder

cancer (MIBC) or later they progress to MIBC. It is extremely rare below 30 years of age and only limited case studies are reported where bladder cancer was diagnosed in young adult and paediatric patients. Various chemical carcinogens have been identified which are responsible for bladder cancer, out of which cigarette smoking is responsible for near about 50% of cases and 20% of cases are caused by occupational exposure.

2.Case Report

A 36 year old gentleman with no co-morbidty with Eastern Cooperative Oncology Group Performance Status 1 (ECOG PS - I) presented to our clinic with post [TURBT] Trans Urethral Resection of Bladder Tumor procedure. He had a history of occasional hematuria for the last 15 days. His home town is located in a remote village which is far away from the city. He is a farmer by occupation and his 3 generations were involved in farming business. No relevant family, past, surgical and medical history. He had no addiction. He consulted a nearby local general practitioner for hematuria and he had been advised ultrasound of abdomen with pelvis. Ultrasound was suggestive of bladder tumor of size 3x2 cm at postero-lateral wall of urinary bladder. With this report, he had been referred to urologist. Urologist advised him to do Contrast Enhanced Computed Tomography [CECT] of thorax, abdomen and pelvis. CECT picked up a lesion of size 2.8x2.2 cm located at left postero-lateral wall of urinary bladder with extension at left vesico-uretric junction with no uretric obstruction. No pelvic or retroperitoneal lymphadenopathy with no distant metastasis. He had been advised TURBT and he underwent the procedure. Post TURBT histopathology demonstrated a high grade muscle invasive urothelial carcinoma with carcinoma in situ component at fundus of bladder and prostatic urethra.

Patient was visited to our clinic with the report. Systemic examination was unremarkable. Case was discussed in our Institutional Multidisciplinary Tumor Board and plan was decided to go ahead with upfront surgery. Board advised him a surgical procedure of Radical Cysto-prostatectomy with bilateral pelvic lymph node dissection with ileal conduit or neo bladder. Patient had opted for ileal conduit. He underwent the surgical procedure (Figures 1, 2). Prostate was also removed along with the specimen as prostatic urethra had focus of carcinoma. Ileal conduit was prepared for urinary diversion (Figure 3). From 3rd postoperative day, oral feeding was started. Post operative course was uneventful and he was discharged on 8th postoperative day. Final histopathology was suggestive of high grade residual urothelia carcinoma of size 2x2 cm with thickness 1 cm located at left postero-lateral wall with invasion into the serosal fat. Prostate, seminal vesicles, bilateral uretric and urethral cut margins were free from tumor and bilateral pelvic lymph nodes (right -0/6, left 0/11) were free from metastasis. Case was re-discussed in the tumor board and adjuvant chemotherapy had been advised to him. He had completed chemotherapy without any major adverse effects. Now he is in follow up with us as per our institutional follow up protocol and after 1 year of completion of treatment, he is still

disease free.

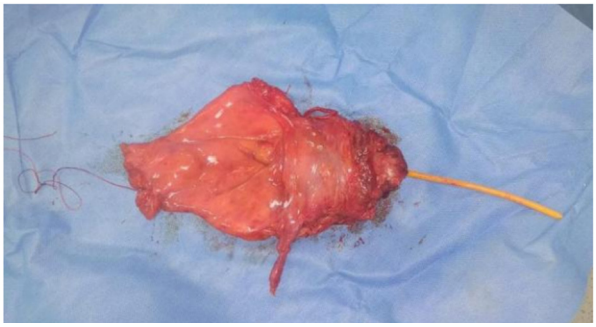


Figure 1. Radical Cystectomy specimen (Dorsal View)

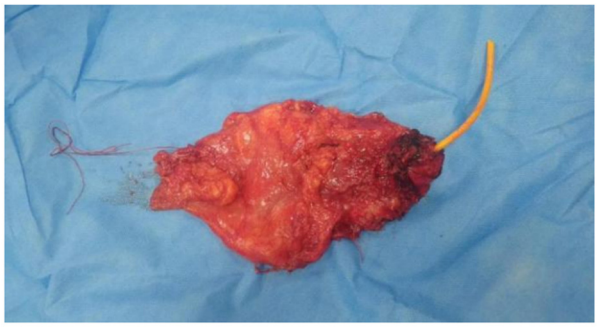


Figure 2. Radical Cystectomy specimen (Ventral View)

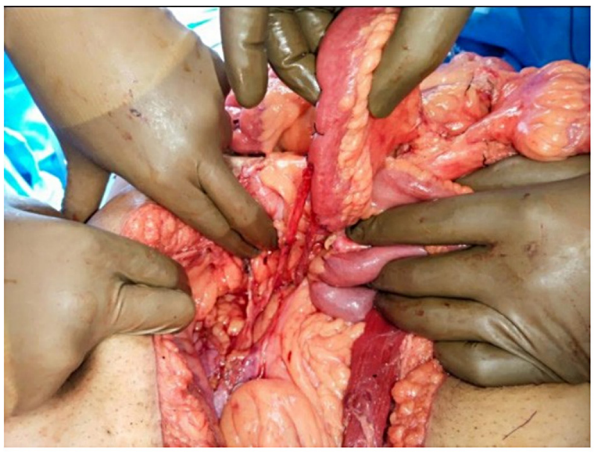


Figure 3. Ileal Conduit with uretric transposition

3.Discussion

The incidence of urothelial bladder cancer (UBC) is 15-20 times higher in people with age above 70 years as compared to people with age range between 30-50 years. The incidence is 15-20 times more in males as compared to females. The higher prevalence in males is because of smoking habits and higher occupational exposure to risk factors. Most of the time, patients with UBC present with hematuria which may be painless and macroscopic. Hence, there will be a diagnostic delay for a period of 6-12 months. Utrasound imaging is reliable and most sensitive tool for detecting UBC. Urine cytology has very low sensitivity and it carries less important role in diagnosing UBC. Computed Tomography is useful in assessing upper urinary tract and distant metastatic foci. Several case studies reported that UBC below 20 years of age has different clinical and pathological features as compared to others. As the age increases, the incidence of high grade UBC increases along with it and in young patients it is low stage and low grade. According to Wang, the 5-year survival of UBC is better in young patients (93.8%) as compared to older people (85.1%). The index case presented with occasional painless hematuria. However, he reported immediately to the treating medical team after noticing it and underwent the investigations and procedure as suggested.

Several studies were conducted to find out the genetic alterations responsible for development of UBC in young patients who does not have any risk factors and presented with lower stage and low grade tumors. Wild et al and Owen et al reported that genetic alterations are extremely rare under 20 years

of age. The recurrence rate of UBC is less in younger individuals as compared to elderly people. Na et al. Reported the recurrence rate of 7.1% in patients below 40 years of age as compared to 38% in patients with age above 60 years. Most of the young patients present with UBC with non-muscle invasive disease with lower progression, low grade and lower recurrence rate. Paner et al. demonstrated in his review of younger patients with UBC with age below 30 years, only 3.0% had muscle-invasive disease and only 1.7% had high-grade tumor. However, aggressive bladder cancer has been reported in children - a 31-month- old and a 14-year-old. The index case had no known risk factors and he had been diagnosed with high grade disease at the time of initial diagnosis.

Cigarette smoking is by far the most prevalent risk factor for developing UBC. Polycyclic Aromatic Hydrocarbon (PAH) exposure is responsible for 15- 20% of bladder cancer cases. In rest of the cases, other occupational carcinogens and genetic alterations are the causative agents. Radical cystectomy is the definitive curative treatment option for patients with muscle invasive bladder cancer, recurrent high grade superficial bladder cancer and high-grade T1 disease. In the postoperative period, young patients may suffer from infertility and impotence. Nerve sparing surgery along with preservation of prostate and seminal vesicle should be an option in young patients. Neobladder urinary diversion is the preferred surgical option which helps in maintaining body image and quality of life. In the index case, prostatic urethra was involved by the disease, so he underwent prostatectomy and he does not have issue of impotency in the postoperative period. Thus, it is a rare presentation of high grade muscle invasive bladder cancer without any known risk factors in a 36 year old gentleman.

4.Conclusions

Bladder cancer oncogenesis is still unclear in young adults due to lack of precise research studies. High grade muscle invasive bladder cancer is rare below 40 years of age and preservation of fertility with maintenance of quality of life are prime important factors while doing radical cystectomy in young patients.

Disclosures

Human subject

Informed consent was obtained from the patient for being included in the study.

References

[1]Yossepowitch O, Dalbagni G. Transitional cell carcinoma of the bladder in young adults: presentation, natural history and outcome. J Urol 2002;168:61-66.

[2]The Global Cancer Observatory ; March, 2021; Glo-bocan 2020.

[3]https://gco.iarc.fr/today/data/factsheets/populations/356-india-fact-sheets.pdf.

[4]Rogers, C. G., G. S. Palapattu, S. F. Shariat, P. I. Karakiewicz, P. J. Bastian, Y. Lotan, et al. 2006. Clinical outcomes following radical cystectomy for primary nontransitional cell carcinoma of the bladder compared to transitional cell carcinoma of the bladder. J. Urol. 175:2048-2053; discussion 2053.

[5]Ghoneim, M. A., M. M. el-Mekresh, M. A. el-Baz, I.A. el- Attar, and A. Ashamallah. 1997. Radical cystectomy for carcinoma of the bladder: critical evaluation of the results in 1,026 cases. J. Urol. 158:393-399.

[6]El-Bolkainy, M., N. Mokhtar, M. Ghoneim, and M.Hussein. 1981. The impact of schistosomiasis on the pathology of bladder carcinoma. Cancer 48:2643- 2648.

[7]Kassouf, W., P. E. Spiess, A. Siefker-Radtke, D. Swanson, H. B. Grossman, A. M. Kamat, et al. 2007. Outcome and patterns of recurrence of nonbilharzial pure squamous cell carcinoma of the bladder. Cancer 110:764-769.

[8]Zuiverloon TC, Abas CS, van der Keur KA, Vermeij M, Tjin SS, van Tilborg AG, et al. In- depth investigation of the molecular pathogenesis of bladder cancer in a unique 26-year old patient with extensive multifocal disease: a case report. BMC Urol. 2010; 10: 5.

[9]Yossepowitch O, Dalbagni G. Transitional cell ca-cinoma of the bladder in young adults: presentation, natural history and outcome. J Urol. 2002; 168: 61- 66.

[10]Lerena J, Krauel L, Garcia-Aparicio L, Vallasciani S, Suñol M, Rodó J. Transitional cell carcinoma of the bladder in children and adolescents: six-case series and review of the literature. J Pediatr Urol. 2010; 6:481-485.

[11]Stanton ML, Xiao L, Czerniak BA and Guo CC: Urothelial tumors of the urinary bladder in young patients: A clinicopathologic study of 59 cases. Arch Pathol Lab Med 137: 13371341, 2013.

[12]Fleshner NE, Herr HW, Stewart AK, Murphy GP, Mettlin C, Menck HR. The National Cancer Data Base report on bladder carcinoma. The American College of Surgeons Commission on Cancer and the American Cancer Society. Cancer 1996;78:1505- 1513.

[13]Brookfield KF, Cheung MC, Gomez C, Yang R, Nie- der AM, Lee DJ, Koniaris LG. Survival disparities among African American women with invasive bladder cancer in Florida. Cancer 2009; 115: 4196-209.

[14]Farahmand M, Almasi-Hashiani A. Epidemiology of bladder cancers in fars province, Southern Iran(2003-2008). J Kerman Uni Med Sci 2013; 20: 387-394.

[15]Wang QH, Ji ZG, Li HZ, Fan H, Chen ZG, Shi BB, Fang Y. Clinicopathologic Comparison of Urotheli-al Bladder Carcinoma in Young and Elder Patients. Pathol Oncol Res 2016;22:67-70.

[16]Wild P, Giedl J, Stoehr R, Junker K, Boehm S, van Oers JM, Zwarthoff EC, Blaszyk H, Fine SW, Humphrey PA, Dehner LP, Amin MB, Epstein JI, Hart-mann A. Genomic aberrations are rare in urothelial neoplasms of patients 19 years or younger. J Pathol 2007;211:18-25.

[17]Owen HC, Giedl J, Wild PJ, Fine SW, Humphrey PA, Dehner LP, Amin MB, Epstein JI, Blaszyk H, Hughes D, Hartmann A, Stoehr R, Catto JW. Low frequency of epigenetic events in urothelial tumors in young patients. J Urol 2010;184:459-463.

[18]Na SW, Yu SH, Kim KH, Hwang EC, Jung SI, Kwon DD, Kang TW. The prognosis of patients less than 40 years with bladder cancer. J Cancer Res Ther 2014;10:710-714.

[19]Paner GP, Zehnder P, Amin AM, Husain AN, Desai MM. Urothelial neoplasms of the urinary bladder occurring in young adult and pediatric patients: a comprehensive review of literature with implications for patient management. Adv Anat Pathol. 2011; 18: 79-89.

[20]Lezama-del Valle P, Jerkins GR, Rao BN, Santana VM, Fuller C, Merchant TE. Aggressive bladder carcinoma in a child. Pediatr Blood Cancer. 2004; 43: 285-288.

[21]Scott AA, Stanley W, Worsham GF, Kirkland TA Jr, Gansler T, Garvin AJ. Aggressive bladder carcinoma in an adolescent. Report of a case with immunohis-tochemical, cytogenetic, and flow cytometric characterization. Am J Surg Pathol. 1989; 13: 1057-1063.

Evaluating the Effect of Irisin on Obesity-Concerning Physical Activity

Onur Oral(1) Pınar Tatlibal(2) Evangelia Stavropoulou(3)
1.Faculty of Sports Sciences, Ege University, Izmir, Turkey
2.Necat Hepkon Faculty of Sports Sciences, Dokuz Eylül University, Izmir, Turkey
3.Research in Information and Communication, University of Lorraine, France

ABSTRACT

Background: The study aims to investigate and evaluate the impact of irisin on physical activity and obesity. Materials and Methods: In the search for scientific literature related to this review the US National Library of Medicine (PubMed) used MEDLINE and SportDiscus data and the terms “irisin”, “physical activity”, and “obesity”, were used. The relevant literature has also taken its source from the research of relevant articles from reference lists derived from data studies. Results: Irisin, an emerging myokine in the scientific community, has received high attention as a potential contributor to obesity. This hormone is also associated with physical activity. Conclusions: Irisin was recently identified as a myokine known to respond to physical activity. Adequate recognition of this hormone may play an active role in the prevention and treatment of obesity.
Keywords: Physical activity;Irisin;Obesity

1.Introduction

Obesity is a global public health problem in several countries. Mexico and the U.S are the countries with the highest rates of obesity . Due to the positive effects of physical exercise and activities on the prevention of obesity, it is important that exercise is an indispensable part of a healthy life. The sedentary lifestyle, which occurs when children and adolescents who were born and grew up in the digital age, that is, intensively use modern age technologies such as the internet and smart phones, tablets, computers, spend excessive time on computers and information technologies, can increase the risk of obesity in children and dolescents .

Irisin was recently identified as a myokine that is known to be responsive to physical activity. In their study, Boström et al. introduced irisin to the world of science as a PGC-1α-dependent myokine. Irisin affects white adipose tissue to stimulate UCP1 expression in mitochondria and enhances thermogenesis. Data state that irisin causes white fat to become brown, thus provoking mitochondria to burn more of the stored fat.

Irisin is a myokine that was newly revealed in the world of science. According to the latest studies, cytokines and other peptides play important roles in multifactorial pathogenic mechanisms concerning obesity.

Irisin has received high interest as a potential contributing factor to obesity. Irisin,

a hormone, is secreted from fibronectin type III domain-containing protein 5 (FNDC5), which is found in skeletal muscle.

Irisin is viewed as an attractive potential curative element for obesity and metabolic disorders. Nonetheless, the physiological determinants of irisin and its secretion form are not yet known exactly.

2.Discussion

In a research study of Boström et al. add further information about irisin and in particular that irisin promotes mitochondrial biogenesis which improves fat metabolism. In addition, according to the mice study, when irisin level increases, more calories are spent regardless of the physical activity level. Another result of the study is that regular physical activity increases irisin levels, suggesting an exercise period of 3 weeks for mice and 10 weeks for humans. Boström and colleagues conducted a study on mice. The research resulted in increased irisin levels measured up to 65% after 3 weeks of swimming exercise. A similar human study by these investigators indicated consistent results as irisin levels increased after endurance exercise of 2.5 months [15].

The amount of irisin release from adipose tissue was determined to be lower in lean rats than in obese rats.

Short-term endurance exercise triggered the release of irisin by sc and visceral adipose tissue in rat studies. Similarly in a study that was conducted on human subjects, acute aerobic training increased circulating irisin but only transiently.

In one study targeting pigs, however, no effect of exercise on FNDC5 gene expression was found. But however, another study found that exercise increased irisin release in mice and humans.

A study by Stengel et al. determined lower plasma irisin levels in people with anorexia and a linear association between irisin and BMI.

Pardo et al. claimed that for every 1kg increase in fat mass, there is a twofold increase in irisin. And research on adults demonstrated that FNDC5/irisin secretion increases after short periods of endurance training. And in another research in obese subjects determined an increase of irisin levels by 12% after 12 months of lifestyle change. However, they weren't found associated with BMI alterations.

Irisin is a recently discovered hormone. It is reported to have an important role in energy homeostasis and obesity [23-25]. Results of a study demonstrated a positive relationship between irisin and BMI, fasting blood glucose, TG, and diastolic BP. In addition, the study indicated a negative relationship between irisin and circulating HDL cholesterol.

3.Conclusions

It is possible to come across many scientific publications that physical activity is a very effective method among many methods for the healthy regulation of energy balance in the treatment process of the obesity problem, which is increasing its impact all over the world due to a sedentary life and unhealthy eating habits. With a more comprehensive examination of this positive effect in terms of the irisin hormone, the importance of this hormone for a healthy life will be understood more clearly.

It is known that a physically active life is very import- ant for a healthy life. The habit of regular exercise, which increases the secretion of irisin and some other hormones, especially increases muscle endurance, muscle strength, muscle flexibility, as well as prevents obesity (excessive weight), helps maintain weight, reduces the risk of cardio- vascular disease, regulates sleep quality, increases bone mineral density, increases blood flow. It has been shown that it contributes to the reduction of fat and glucose levels, thus reducing the incidence of certain types of cancer and chronic health complaints that may occur with aging.

Acknowledgement

We would like to express our special thanks of gratitude to George N. NOMIKOS for his very successful contribution to the literature research process and unique academic support in the publication during the process of this review article.

Conflict of Interest

The author certifies that there is no conflict of interest with any financial organization regarding the material dis- cussed in the manuscript.

Funding

The author certifies that there is no funding from any financial organization regarding the material discussed in the manuscript.

Author's Contributions

All authors read and approved the final version of the manuscript.

References

[1]Elder, J.P., 2013. Mexico and the USA: the world's leaders in the obesity epidemic. Salud Pública México. 55, 355.

[2]González-Gross, M., Gómez-Lorente, J.J., Valtueña, J., et al., 2008. The "healthy lifestyle guide pyramid" for children and adolescents. Nutrición hospitalaria. 23(2), 159-168.

[3]Oral, U., 2020. Political Perception Of Generation Z In Turkey. International Journal of Advanced Science and Technology. 29(11s), 493-500.

[4]Martinez-Gomez, D., Moreno, L.A., Romeo, J., et al., 2011. Combined influence of lifestyle risk factors on body fat in Spanish adolescents-the Avena study. Obesity Facts. 4(2), 105-111.

[5]Boström, P., Wu, J., Jedrychowski, M.P., et al., 2012. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature. 481, 463-468.

[6]Kraemer, R.R., Shockett, P., Webb, N.D., et al., 2014. A transiently elevated irisin blood concentration in response to prolonged, moderate aerobic exercise in young men and women. Hormone and Metabolic Research. 46(2), 150-154.

[7]Wu, J., Boström, P., Sparks, L.M., et al., 2012. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell. 150, 366-376.

[8]Pedersen, B.K., Febbraio, M.A., 2012. Muscles, ex- ercise, and obesity: skeletal muscle as a secretory organ. Nature Reviews Endocrinology. 8(8), 457-465.

[9]Elizondo-Montemayor, L., Silva-Platas, C., Tor- res-Quintanilla, A., et al., 2017. Association of Irisin Plasma Levels with Anthropometric Parameters in Children with Underweight, Normal Weight, Over-weight, and Obesity. BioMed Research International. 2628968.

DOI: https://doi.org/10.1155/2017/2628968

[10]Jedrychowski, M.P., Wrann, C.D., Paulo, J.A., et al., 2015. Detection and quantitation of circulating human irisin by tandem mass spectrometry. Cell Metabolism. 22(4), 734-740.

[11]Anastasilakis, A.D., Polyzos, S.A., Saridakis, Z.G., et al., 2014. Circulating Irisin in Healthy, Young Individuals: Day-Night Rhythm, Effects of Food Intake and Exercise, and Associations With Gender, Physi- cal Activity, Diet, and Body Composition. The Journal of Clinical Endocrinology & Metabolism. 99(9), 3247-3255. DOI: https://doi.org/10.1210/JC.2014-1367

[12]Moreno-Navarrete, J., Ortega, F., Serrano, M., et al., 2013. Irisin is expressed and produced by human

muscle and adipose tissue in association with obesity and insulin resistance. The Journal of Clinical Endo- crinology and Metabolism. 98, E769-E778.

[13]Polyzos, S.A., Kountouras, J., Shields, K., et al., 2013. Irisin: a renaissance in metabolism? Metabo- lism. 62, 1037-1044.

[14]Boström, P.A., Fernández-Real, J.M., Mantzoros, C., 2014. Irisin in humans: recent advances and questions for future research. Metabolism. 63, 178-180.

[15]Saraslanidis, P., Petridou, A., Bogdanis, G.C., et al., 2011. Muscle metabolism and performance improvement after two training programmes of sprint running differing in rest interval duration. Journal of Sports Sciences. 29, 1167-1174.

[16]Roca-Rivada, A., Castelao, C., Senin, L.L., et al., 2013. FNDC5/irisin is not only a myokine but also an adipokine. PLoS ONE. 8(4), e60563.

[17]Huh, J.Y., Panagiotou, G., Mougios, V., et al., 2012. FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. Metabolism: Clinical and Experimental. 61(12), 1725-1738.

DOI: https://doi.org/10.1016/j.metabol.2012.09.002

[18]Norheim, F., Langleite, T.M., Hjorth, M., et al., 2014. The effects of acute and chronic exercise on PGC-1α, irisin, and browning of subcutaneous adipose tissue in humans. Federation of European Biochemical Societies Journal. 281, 739-749.

[19]Fain, J.N., Company, J.M., Booth, F.W., et al., 2013. Exercise training does not increase muscle FNDC5 protein or mRNA expression in pigs. Metabolism. 62, 1503-1511.

[20]Stengel, A., Hofmann, T., Goebel-Stengel, M., et al., 2013. Circulating levels of irisin in patients with anorexia nervosa and different stages of obesity—correlation with body mass index. Peptides. 39(1), 125- 130.

[21]Pardo, M., Crujeiras, A.B., Amil, M., et al., 2014. Association of Irisin with fat mass, resting energy ex- penditure, and daily activity in conditions of extreme body mass index. International Journal of Endocrinology. Article ID 857270, 9 pages.

[22]Blüher, S., Panagiotou, G., Petroff, D., et al., 2014. Effects of a 1-Year Exercise and Lifestyle Intervention on Irisin, Adipokines, and Inflammatory Markers in Obese Children. Obesity.

DOI: https://doi.org/10.1002/oby.20739

[23]Park, K.H., Zaichenko, L., Brinkoetter, M., et al., 2013. Circulating irisin in relation to insulin resistance and the metabolic syndrome. The Journal of Clinical Endocrinology and Metabolism. 98(12),4899-4907.DOI: https://doi.org/10.1210/jc.2013-2373

[24]Timmons, J., Baar, K., Davidsen, P., et al., 2012. Is irisin a human exercise gene? Nature. 448, E9-E10.

[25]Villarroya, F., 2012. Irisin, turning up the heat. Cell Metab. 15, 277-278.

[26]Liu, J.J., Wong, M.D., Toy, W.C., et al., 2013. Lower circulating irisin is associated with type 2 diabetes mellitus. Journal of Diabetes and Its Complications. 27, 365-369.