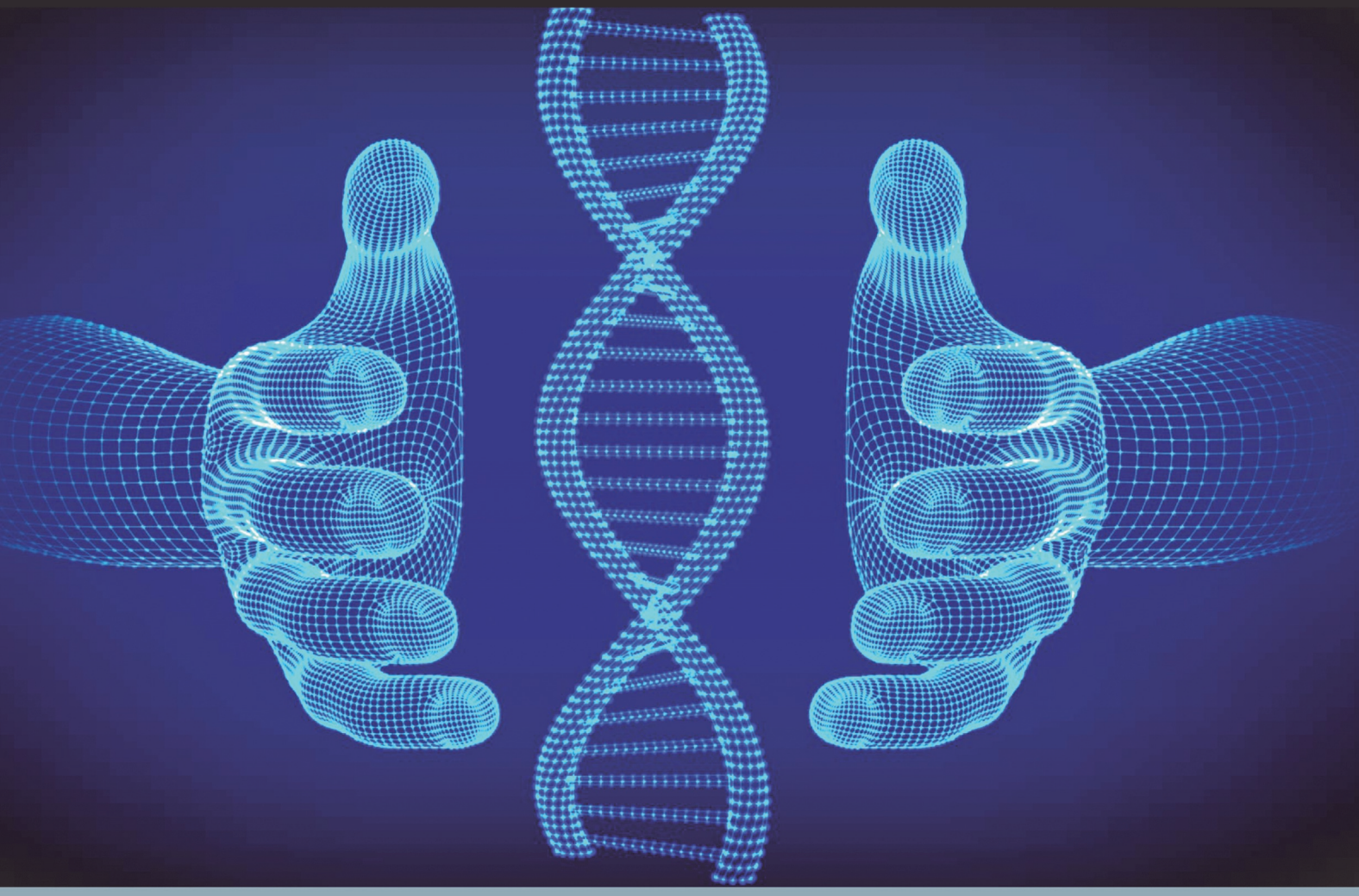
Personalized Medicine JOURNAL

Medical Journal/7 year/No,25/150000Rials/2022 Spring/ ISSN 2717-3860



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Journal Information

Name	Personalized Medicine Journal
Abbreviated name	PMJ
Date of first issue published	February 2019
Concessionaire	AmitisGen Med TECH Group
Release period	Quarterly

Editorial Board Information

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Personalized Medicine Journal
Spring 2022,Volume7,Issue 25
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http://pmjournal.ir Review Article

Spring 2022,Volume 7, Issue 25(1-14)

Epigenetic as a Novel Biomarker Associated with PAH Exposure and Breast Cancer Risk

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DOI: 10.22034/pmj.2022.253549

Submitted: 2022-02-21
Accepted: 2022-05-23

Keywords:
Breast Cancer
DNA methylation
Non-coding RNAs
Personalized Medicine

Abstract:
The pathophysiology and molecular pathways of breast cancer (BC) are still unclear, but it appears that BC is caused by the interaction between genetic susceptibility and environmental factors. Epidemiology studies have shown the increase risk of BC through polycyclic aromatic hydrocarbons (PAH) exposure. Environmental carcinogens induce disease pathways by altering the expression of specific genes that may be a consequence of epigenetic modifications. In order to understand the effects of PAHs in the BC risk, the epigenetic pathway may consider as an important key and likely play a role in BC initiation. Novel epigenetic biomarkers and treatments hold promise in the approch of personalized medicine. Here, we focus to review the epigenetic factors in relation to polycyclic aromatic hydrocarbons exposure that may influence BC risk.

INTRODUCTION

Overview of epigenetic modifications in BC Susceptibility

Breast cancer (BC) is the second malignancy that leads to death in women. It is heterogeneous with variable biological features on the molecular level(1). BC is considerably curable and outcomes improve in patients with early-detection, non- metastatic disease (2). Several factors such as age, family history, late-age pregnancy, early menarche, and late-onset menopause(2), high levels of estrogen during pregnancy(3), lack of breastfeeding, and alterations in gene expression patterns(4) can affect BC risk. Other factors that influence BC risk include: diet, life-style, and BC susceptibility genes (2). Remarkably, the accumulation of multiple, low- penetrant mutations can increase breast cancer risk(2, 5).

Various epigenetic abnormalities are present in BC. Epigenetic alterations including hypermethylation of DNA damage response genes (BRCA1 and hMLH1), cell cycle regulation and apoptosis (CCND2, CDKN2A/p16, RASSF1A, APC), p14(ARF), CDH1, MGMT, GSTP1, and cell signaling (ER and RARβ 2) are important in breast carcinogenesis(6) and elevate the risk of BC(7). Epigenetic modifications in ATM, MLH1, MSH2, and PALB2 are germline (8) and sometimes they carry with other germline mutations. Promoter hypermethylation of PALB2 was found in women who are the carrier for BRCA2 germline mutations (9). Multiple-case BC families with no identified genetic mutation may be caused by heritable DNA methylation marks(8). Heritable epigenetic in BC susceptibility genes which are derived from PBMC may be used as a guidance biomarker to predict BC risk with the unknown mutation. The normal breast tissue adjacent to the tumor location may show genes with promoter hypermethylation, such as RUNX3 and RASSF1A. The results indicate that the aberrant epigenetic modifications occur in the early stage of BC, and premalignant epigenetic alterations may be widespread from the tumor epicenter to the surrounding cells (10, 11).

Histone acetylation and methylation associate with BC(12-15). The studies demonstrated that acetylation of H3 lysine enhance in BC cells(16). The increase of the H4 acetylation can lead to abnormal expression of DNMT1 (17). Histone acetylation play important role in DNA damage response and determines the efficiency of DNA repair(18). Furthermore, HDAC1 can encourage cell proliferation by increasing the expression

of two LncRNAs, BC01600 and AF116637, in cancer tissue(19). Aberrant expression of the ncRNAs and their interaction with epigenetic proteins is shown in BC(20).

The level of epigenetic components such as methyltransferase (DNMT)-1 is increased in lymph node metastasis while DNMT 3a and 3b are elevated in severe stages. Other enzymes involved in epigenetic modifications are ten–eleven translocation (TET) family of methylcytosine dioxygenases. TET1 is inhibited by miR29 and subsequently, affects chromatin remodeling. In the progression steps of BC, TETs expression is decreased. Upregulation of TET1 promotes the expression of HOXA7/9 (21). Petr Novak and et al reported that the HOXA gene expression including HOXA1 to HOXA10 significantly decreases in BC tissue. This small epigenetic region is located at gene family clusters and inactivates by epigenetic microdeletions in BC (22).

PAH and breast cancer

Interaction between environment and genetics plays an important role in tumorigenesis, and its evaluation improves the prediction of BC risk (23-27). Although a recently cohort study in China observed that there is not a correlation between BC and Indoor solid fuel combustion(28,29), Epidemiologic and experimental studies demonstrate that PAH exposure is associated with an increase in breast cancer risk (30,34-37). PAH a widespread chemical carcinogen in ambient environment pollution, enter the human body from a variety of sources including smoke from cigarettes and tobacco, inhaling gasoline and diesel-fueled, combustion of coal and coke, and eating grilled and smoked meats(38). These lipophilic compounds have been stored in breast fat and metabolized in breast epithelial tissues to deleterious state. So in this way, it can affect cellular morphology, division, and growth (39, 30). PAH biotransformation can bind to the DNA, forming covalent PAH-DNA adducts and also react with proteins such as hemoglobin and albumin (18, 31). DNA adducts can alter promoter methylation leading to abnormal gene expression, and ultimately tumorigenesis(32). PAH-DNA adducts reflect exposure to PAH, and they are presumed as an early effect marker for cancer(26). The level of PAH-DNA adducts is high in women who are at high risk due to cancer family history. The population-based cancer registry reports that early exposure to environmental factors, when the breast cells divide quickly, can enhance BC susceptibility (40, 24-26). A meta-analysis of 15 cohort studies showed that BC incidence is high among current and former smokers women, and there is an association between BC and active smoking(41). Two population-based case-control studies, demonstrated the association between residential exposures to PAH and BC risk(35-37). DNA repair capacity in response to PAH-DNA adducts can affect BC risk. Results showed an association between PAH-DNA adducts and BC risk in women who have a variation in in ERCC2 and XRCC1(DNA repair genes)(35). An invitro study showed that PAH effects on gene expression in ER-positive cell line(42).

In addition to genetic alterations, aberrant epigenetic patterns lead to the activation of oncogenes, downregulation of tumor suppressor genes, and reduction of DNA repair capacity, which are involved in cell transformation(43, 44). Epigenetic changes are involved in BC initiation and progression without changing DNA sequences(45). The germline mutation of high penetrance genes such as BRCA1/2 was a landmark biomarker for familial BC prediction, but the majority of BC occurs in women who don't have any germline mutation. In sporadic breast tumors, which constitute the main of BC cases, epigenetic mechanisms are involved in the downregulation of BRCA1 in the absence of mutations in the BRCA1 gene. Furthermore, in women who carry a mutated BRCA1 gene, silencing the wild allele by epigenetic modification may further enhance the BC risk(46). DNA methylation, histone modification, and non-coding RNA are the most epigenetic events that regulate the gene expression (43, 44). PAHs are capable of epigenome modification. PAH exposure through epigenetic modification may affects the telomere length shortening(47). Treatment of human cells with BaP or related metabolites causes promoter hypo- and hypermethylation and histone modifications, subsequently changes the expression of critical genes in cancer. (48). PAH alters DNA methylation in a number of genes through noncoding RNA (49). Methylation of genes are associated with the presence of PAH adducts in breast and breast tumor (50,51). Furthermore, Activation of AhR by PAH, which is initially bound to the HSP90, XAP2 and the HSP90-associated co-chaperone p23 protein, induces dimerization with ARNT. This process leads to the expression of the enzymes that are involved in a variety of functions, including chromatin structure remodeling, cell proliferation, loss of cell adhesion, and inflammation. (32).

The achievement of a biomarker for detecting cancer at an early stage is one of the main priorities in cancer research. Cancer biomarkers should have sensitivity and specificity in the clinical application level. Epigenetics can serve as a biomarker of the interaction between genetic predisposition and past and current environmental exposures. Such biomarkers can help to design preventive strategies and diagnose at-risk subgroups and personalized medicine (52). However it needs large-scale epidemiological Studies. Nowadays, blood-based epigenetic biomarkers have become an attractive and controversial subject for the assessment

of solid tumors. It should be noted that this kind of biospecimen cannot precisely reflect the modification of target tissue(some circulating molecules such as cell-free DNAs and RNAs (cfNAs), exosomes and non-coding RNAs, which originate from cancerous cells or solid tumors can present valuable information about tumor cells). In this regard, extensive efforts have been made to detect epigenetic biomarkers for the early diagnosis of BC(53). Therefore, to gain better insights on the effects of PAHs in the BC risk, the epigenetic pathway may consider as an important key. The studies of PAH-associated epigenetic alterations in particular susceptibility loci may provide valuable insight into the underlying biological mechanisms in PAH-associated carcinogenesis. In this review, we describe how PAHs influence the epigenetic modifications including DNA methylation, histone modification, and non-coding RNA and explain how specific epigenetic modifications might increase susceptibility to BC risk.

DNA Methylation

Methylation alteration at specific genes

DNA methylation is one of the epigenetic processes. PAH exposure has a potential role in gene silencing through hypermethylation at specific loci cancer(50, 51)and global DNA hypomethylation (54). Mammary cells can store and concentrate lipophilic aromatic hydrocarbons in breast fat. These cells metabolize the aromatic hydrocarbons into DNA-binding metabolites(55). Binding the PAH metabolites to nitrogenous bases can produce DNA adducts. Methylation of CpG islands increases DNA binding to reactant electrophiles(56-58). It has been shown that methylated CpG sequences at the exon 7 of the p53 gene increase the BPDE binding constant to G in codon 248(56). The linear regression model shows that DNA adducts formation is higher in CpG islands(58). Methylation of promoter CpG islands is associated with the repressive histone markers, including trimethylation of histone 3 at lysine 27 and 9. Cytosine methylation within the promoter can identify by methyl group-binding proteins (MBDs). finally, gene silencing promotes by recruiting co-repressor complexes with histone modification enzymes such as deacetylases (HDACs) (59). It is noteworthy that documents indicate that histone modifications is more rapid response to environmental effects, but DNA methylation mediates gene silencing over a longer time frame(60, 61).

Study the genome-wide DNA methylation in mice lung tissue has been shown that the promoter region of Pten and Tpd52l1 are respectively hyper- and hypomethylated, in DBC exposure(62). PTEN is one of the most frequently mutated genes identified in DCIS and IDC subtypes with hypermethylation(63). The meta-analysis study introduces it as a valuable biomarker for the diagnosis of BC(63). Tpd52l1 belongs to the tumor protein D52 gene family and is implicated in BC(64). Tpd52 family plays a role in cell proliferation and calcium signaling. It can be assumed that this result may not show methylation patterns for all tissues, but PAH may also influence the genes mentioned in breast tissue.

BaP can modify BRCA1 methylation patterns in ER+ BC (MCF-7) cells(42). Study of tumor tissue biopsy from patients with invasive or in situ breast cancer revealed that BRCA1 promoter methylation was high among invasive cancers and premenopausal cases and it frequently elevates mortality(65). Breton and et al. found that methylation patterns of receptor tyrosine kinase and receptor tyrosine phosphatase change as a result of maternal smoking(66). Another study in adolescents who were exposed to cigarette smoke in during pregnancy showed that methylation was enhanced in the promoter and 5'UTR(untranslated region) of brain-derived neurotrophic factor(67). In contrast, a cross-sectional study conducted in PAH-exposed brick makers, DNA methylation of P53 and IL-12 was inversely associated with PAH exposure(68). Study in animal models revealed that prenatal PAH over-exposure leads to an increase in the PPARγ expression in offsprings which regulate through the decrease in DNA methylation. Activation of peroxisome proliferator-activated receptor γ (PPARγ) plays an essential role in the proliferation and invasion of breast cancer cells(69). Therefore, through enhancement of the PPARγ via epigenetic alteration, PAH may increase BC risk. Promoter methylation of APC and RAR-β genes, as well as PAH-DNA adducts, elevate the risk subtype of ER+/PR+ BC (50). Additionally, the LIBPCS study found that the source of PAHs can influence the methylation of the specific genes.

PAH exposure is associated with impaired Treg function that is the result of altered methylation of the Foxp3 promoter(60). Kohli and et al. found that tobacco smoke exposure increases methylation of IFN-gamma in T effector cells and FOXP3 in T regulatory. They noted that cigarette smoke, along with other chemicals like nicotine and particulate matters may contribute to epigenetic alterations(70). Treg cell ablation accelerates the tumor progression and increases tumor growth in early-stage, in contrast, Foxp3+ Treg cells accumulate in advanced BC(71). FOXP3 is expressed in a variety of normal and cancerous cells in addition to Treg. FOXP3 downregulation is associated with cancer development in BC. Evidence shows that FOXP3 is a tumor suppressor gene in the breast. HER2 and SKP2, two oncogenes in breast cancer, are repressed by FOXP3. HER-2 protein is a transmembrane receptor for growth factor, which is present in 15-20% of invasive breast cancers. SKP2 has a role in ubiquitination and degradation of the cdk-

inhibitor p27 and its expression increase in 50% of breast cancers(72). So, it may be considered that PAH by epigenetic alteration of FOXP3 in breast tissue and immune cells conducts tumorigenesis.

Alteration at global methylation

Long interspersed nuclear elements-1 (LINE-1) belong to classI retrotransposable elements that are the most abundant elements of classI in the DNA. LINE-1 reactivation can affect cellular functions, such as epithelial cell differentiation programs, cellular adhesion, inflammation, metabolism, induction EMT(73, 74). LINE-1 elements length is 6–7 kb and they have 500000 copies in the human genome. It consists of two open reading frames (ORFs); ORF1 and ORF2, which are involved in duplication and transposition of elements(75). DNA methylation at the 5' position of cytosines by DNA methyltransferases (DNMTs) is an important mechanism for LINE-1 silencing. Although LINE-1 is active during early embryogenesis, its activity is suppressed when cells initiate the differentiation program. The content of PAH-DNA adducts is opposite to global DNA-methylation. Also, there is demonstrated that BaP induces DNA hypomethylation of Short interspersed nuclear elements (SINEs) and Long terminal repeats (LTRs) elements(77). Pavanello and et al analyzed the methylation status of specific genes and repetitive elements including, Alu and LINE-1 in PBLs isolated from coke-oven workers. They reported that the global methylation level in the PAH-exposed group is significantly higher than the controls. Also, the levels methylation of the tumor suppressor including, p53 and HIC1 in Coke-oven workers decrease in comparison to the control group. Moreover, the methylation status of Alu and LINE-1 has a significant positive correlation with anti-B[a] PDE-DNA adduct and 1-pyrenol, conversely, p53 methylation is negatively correlated with 1-pyrenol and anti-B[a] PDE-DNA levels(78). Inconsistent with Pavanello; Ivo Teneng (79) and Boissinot et al (80) Jin Yang (99)showed that LINE-1 methylation is reduced in-vitro and in PAH-exposed individuals, respectively. Ivo Teneng et al demonstrated that BaP exposure enhances transcriptionally active chromatin markers such as H3K4me3 and H3K9ac and reduces the interaction of DNA methyltransferase-1 (DNMT1) with the LINE-1 promoter(79). Also, they explained that the first event that induces by BaP is histone modifications that happen with short term exposure, but long-term impact includes DNA methylation. HDAC1 performs an important role in the proteosomal-mediated degradation of DNMT1(50). Also, they found that LINE-1 hypomethylation response is not just tumor cell-specific, but exists in primary cells. in contrast, Claudia Knothe et al. demonstrated that LINE-1 methylation is a tissue feature(81). Differences in the extent of LINE-1 methylation between these studies may be caused by differences between cell types and length of chemical exposures.

BaP-induced AhR activating regulates LINE-1 expression via the TGFβ/ SMAD2/SMAD3 axis(82). SMAD proteins add four acetyl groups on histone 3 using histone acetyltransferases (HATs) P300 and CBP and induce gene transcription(83). Reduction in global DNA methylation causes genomic instability and initiate the early stage of cancer(84). PAH exposure by decrease LINE-1 methylation may promote its mobility. New somatic LINE-1 insertions have been demonstrated in several epithelial cancers. LINE-1 insertion may lead to the disruption of key cancer genes, and finally, initiation of BC, although it exerts retrotransposition-independent functions(82).

Overall, Repetitive sequences that are extremely methylated, undergo hypomethylation in transformed cells, whereas unmethylated promoter of coding genes (especially tumor suppressors) and noncoding RNA with tumor-suppressor features become hypermethylated and silenced in cancer cells through PAH. Methylation of genes can determine the cancer subtypes. In addition to the type of tissue, the duration of PAH-exposure, the selected method, and study design and capture exposure at the biologically relevant time are also the effective factors in carefully examining the level of methylation of genes. Although PAH- related DNA methylation seems to play a critical role in the initiation of BC pathophysiology, limitations such as, lack of highly exposed and unexposed populations for comparisons, and using blood samples or non-breast cell lines that may have a different response with breast cells, can influence the validation of the results. Moreover, studies have failed to establish clear relations between PAH adducts and hypermethylation or hypomethylation, suggesting that other mechanisms may be involved.

Histone Modification

Histone acetylation

Decrease histone acetylation

Histone modifications modulate the chromatin structure, and subsequently, cause to inhibit or activate gene expression(2, 85). Histone modifications including acetylation and methylation are the most subject studied in breast cancer (86-92). Methylation and acetylation changes are usually found in lysine residues that are present in different positions of histones H3 and H4, whereas phosphorylation changes occur in threonine or serine amino acids (94). Xiangzhi Li and et al reported that MYST1 protein, a histone acetyltransferases, markedly decreased along BaP-induced DNA damage repair in HELF cells. They considered that maintenance of genome integrity is depended of MYST1 level(116).

BaP exposure leads to reduction histone H3k14 acetylation in the StAR promoter region in neonatal animal models which persist to the adult stage. This epigenetic alteration damages sperm count in the long term (95). Sadikovic and et al by invitro studies showed that histone acetylation levels on the promoter region of genes such as MBD2,HDAC1, MBD3, ATRX, and METT5D1 were decreased in BaP-treated cells which are were involved in chromatin remodeling(59).

Increase histone acetylation

The study showed that cigarette smoke condensate (CSC) CSC enhances C/EBP-b level and this henomenon subsequently elevates the expression of LncRNA LOC554202 and miR-31 through increased levels of H3K4Me3 and H3K9/14ac marks in the promoter region of LOC554202(96). Treatment the BC cell lines by BaP leads to hyperacetylation in NAB2, TMF1, BRMS1L, GADD45B, BAX, CYP1B1. These hyperacetylation genes are included cancer-, DNA damage-, transcription-, and detoxification- associated genes(48). CBP/P300 is increased in Cigarette smoke-treated cells and results in the various of acetylating processes(97). Invitro study confirmed that activation of HAT p300 enhances the transcriptional activity of Wnt/β-catenin target genes which is correlated with BC (98).

Alteration in acetylation level has been found in many human cancer such as renal cell carcinoma prostate , lung , gastric , and BCs(99). There is correlation between DNA methylation and histone deacetylation in H3 and H4, and it is suggested that these modification together may be part of a process for tumorigenesis(43). Chromatin modifications provide appropriate conditions for inhibiting the cellular DNMTs activity. These results lead to reducing in cytosine methylation within the target gene that is visible after one replication cycle. Global histone acetylation pattern of histone 3 at lysine 9(48), lysine27 , and lysine18 are changed by BaP exposure. H3K18ac and H3K27ac are located at TSS of the AhR gene and involve in the regulation of the transcriptional activation(100).

Although histone acetylation and deacetylation is critical in normal growth and development, pattern alterations in result of PAH may contribute in tumorigenesis and be predictive biomarkers for BC risk and its phenotype. Also, most studies are reported base on Invitro investigation or blood sample from patients. Studies of PAH-related histone modification patterns and related component as well as target genes that are regulated with these processes, will provide precise inform about possible carcinogenesis of PAH. However, it is still unclear how modifications trigger in histone.

Histone methylation

Histones can be methylated in lysine and arginine residues by two major families of histone methyltransferases(HMTs). One of the most well-known KMTs is EZH2. This enzymes directly are targeted by miRNAs(101, 102) and LncRNAs(103). miRNAs affect gene expression mainly by regulating KMT expressions(104-107) and act as a tumor suppressor miRNAs. These miRNAs are negative regulators of EZH2. The result of their performance leads to suppress the cell invasion, metastatic tumor phenotype(107), and cell proliferation (101)and versus promote apoptosis(104) and decrease cell viability(105). PAH treatment causes the enhancement of expression of epigenetic genes such as DNMT and EZH2(108). In this regard, PAH may regulate the EZH2 expression through inhibition expression of these miRNAs. upregulation of EZH2 has been found in BC. As will be mentioned in the LncRNA section, recruitment and connection of EZH2 by LncRNAs has been determined in the BC and enhance the methylation.

It is found that H3K36me3 modification elevates at the MGMT and MLH1 gene regions in peripheral blood lymphocytes (PBLCs) Coke oven workers exposed to PAHs(18). Also, it is revealed that H3K36me3 is associated with the DNA damage and Urinary 1-hydroxypyrene (1-OHP). Histone modification occurs in response to the PAH exposure and leads to increase expression of DNA repair genes. Therefore, decreasing H3K36me3 modification during PAH exposure may induced malignancy. Furthermore, the H3K4me3 modification negatively correlates with the degree of PAH-induced DNA damage. It is suggested that modifications of H3K4me3 may be involved in the regulation of DNA repair (18), but Genes mediating DNA damage response is unknown.

Co-exposure to PAH and other carcinogens synergistically activate AhR to up-regulate the expression of SUV39H1 methyltransferase and causes to downregulation of SOCS3 ,and subsequently, enhance the Akt and Erk1/2 activation to promote cell transformation(109).

BaP exposure leads to a significantly decreased NR2E3 as well as its target gene, estrogen receptor(110). NR2E3 is a nuclear receptor and regulates transcription of several genes, which involved in development, differentiation, and survival. The expression of NR2E3 causes longer survival in BC patients(111). Tilak Khanal and et al identified that PAH inhibits NR2E3 homodimerization and leads to NR2E3 releasing from ER promoter regions. Then, histone modifications promote through recruiting the LSD1 as a histone demethylase of H3K4me2 and decreasing H4ac in the promoter region of ER(110). These histone modifications are as markers for the

activation transcribed promoter region. It seems that PAHs are not only associated with BC risk but also mediate abnormal epigenetics that will affect BC patients' survival in the future. Collectively, how these post-translational modifications which control diverse chromatin functions can pass to next generation cells is remain unclear. Histone modifications are regulated by various enzymes that may be affected by PAH. Histone abnormalities can impair gene regulation. It is suggested that DNA adduct may play an important role to recruit the histone modification enzyme in CpG islands of promoter regions and enhance the tumor suppressor genes methylation. Depending on the location of histone and types of epigenetic modifications, the PAH impact can have a variety of results.

Histone phosphorylation

Histone phosphorylation by PAH may enhance the target genes transcription which, initiates the tumorigenesis that should experimentally be confirmed. exposure to BaP induce DSBs in mammalian cells which is detectible by Phosphorylation of histone H2AX (γH2AX)(phosphorylated at Ser139) (112). γH2AX is significantly increased in BaP -treated ApcMin/+ cells compared to control, and this assay, confirm an effective genotoxic effect of B(a)P on cells(113). Moreover, several studies demonstrated that exposure of complex mixtures containing PAHs can increase histone phosphorylation markers such as γH2AX, even it is detected in short-term exposure (114-118). Phosphorylation performs by PIKK leads to localize DNA damage response proteins. Then, γH2AX recruits the HATs, HADCs, chromatin -remodeling and –modification complex, and kinase, in sites of DNA damage. In the following, histone H2B is phosphorylated, but H3 phosphorylation on Thr-11 removes by CHK1. This phenomenon is implicated in the regulation of gene expression that is involved in the cell cycle (119). These results indicate that elevation of γH2AX by PAH exposure may reveal mark for aberrant expression of the genes involving in the cell cycle, and implicate in tumorigenesis. tobacco smoke exposure can induce the phosphorylation of HDAC2 on serine/threonine residues and subsequently its degradation carry out with proteasome(120). Scholars reported that lower miR-24-3p expression is associated with plasma BPDE–Albumin adducts. On the other hand miR-24-3p negatively regulates the H2AX(121), thus it may be speculated that PAH by epigeneticaly reduction the miR-24-3p expression promotes DNA-damaging. Wang FP and et al studied the PBLC of coke oven workers. They reported that the H3Ser10 phosphorylation level in the PAH-exposed group is higher than the control group. They also found the correlations between DNA damage and H3Ser10 phosphorylation(122).

However, very few studies have been performed on the role of PAH on histone phosphorylation and BC risk, and frequently research evaluates the histone phosphorylation that is associated with DNA damage. The assess of histone phosphorylation may reflect early PAH-induced DNA damage although, further studies are needed to understand and confirmed the influence of this modification.

Non-coding RNAs(ncRNA)

These group of RNAs are consisted of, micro RNA (miRNA), long noncoding RNA (lncRNA), small-interfering RNAs (siRNAs), piwi-interactingRNA(piRNA), and small nucleolar RNAs. Noncoding RNA have potential to regulate gene expression through different mechanism such as heterochromatin formation, disruption of translation, DNA methylation and histone modification. noncodingRNA cause gene silencing or enhancing expression (123). Aberrant miRNA expressions have been identified in the in BC (124). Beside epigenetic modification in noncoding RNA, it is considered that interaction between noncoding RNA and epigenetic component can promote alteration in gene regulation. Environmental factors can induce binding of LncRNA to histone modification and promote oncogenesis(69). Here we review LncRNA and miRNA(125,126).

Long Non-Coding RNAs

Long noncoding RNAs(LncRNA) are defined as a class of ncRNAs with a dimension of more than 200 nucleotides, which transcript mostly by RNA polymerase II (RNA pol II)(153). Evidence demonstrated that they are involved in a diverse spectrum of biological function in normal cells, so aberrant expression of them can play critical roles in the development of human diseases (127-129). Characteristics including early appearance, stability in biological fluids, tissue specificity have emerged LncRNAs as important biomarkers for health risk assessment (126). Alteration of LncRNA expressions is found in environmental chemical–induced carcinogenesis and cell malignancy (49, 126). LncRNAs are considered one of the essential epigenetic modifiers. LncRNAs interacts with chromatin remodeling complexes and can recruit them to the regulation of gene expression(49, 131).

LncRNA MALAT1

MALAT1 is capable of associating with PRC2 (histone methyltransferase), and subsequently impacts the gene transcripts. MALAT1 binds to the EZH2 subunit and recruit PRC2. PRC2 is composed of EZH2, RBBP4/7, SUZ12, and EED subunits and performs two/trimethylation in histone3 at lysine 27. Similar to MALAT1, LncRNA ANRIL can bind to EZH2(103) and it is suggested that these LncRNAs

may be a scaffold to chromatin-remodeling complex for regulating the expression of genes(132). A study of PBLCs samples from PAHs-exposure workers showed that the expression of MALAT1 was higher in PAHs-exposed group compared to the control group (49). Moreover, this transcript level is elevated in tissue of BC patients. Also, MALAT1 expression levels are correlated with tumor stage and lymph node size and is introduced as a valuable marker of a breast cancer diagnosis (133). This LncRNA is oncogene that could promote proliferation, metastasis, and tumor formation in cells (134-136). Cell-cycle dysregulation leads to uncontrolled cell proliferation that is the main event in cell transformation. Environmental impacts on genes associated with cell cycle may change susceptibility to tumor progression.

LncRNA HOTAIR

HOTAIR, inhibits the expression of p21, via EZH2-mediated histone trimethylation and leads to defection in the cell cycle induced by cigarette smoke extract (CSE)(137). furthermore, the findings confirmed that CSE is involved in malignant transformation through axis IL-6/phospho-STAT3 /HOTAIR(138). In-vitro studies revealed that tri methylation of histone3 at lysine27 , as a biomarker in response to DNA damage, had been upregulated through HOTAIR(100). It is considered that HOTAIR may be presented as a prediction factor of PAH-induced DNA damage and genotoxicity. Moreover, the long intergenic RNA HOTAIR expression in BC cell lines recruits the PCR2 to altered trimethylation on histone H3 lysine 27 , leading to modulating the cancer epigenome and silencing of metastatic suppressor genes(20). The increased expression of HOTAIR has been found in the PAHs-exposed subjects in comparison to the control group (49).

LncRNA TUG1

It has been demonstrated that the TUG1 expression increase in male coke oven workers. This elevation is associated with the level of PAH exposure(49). TUG1 is upregulated in breast cancer and can encourage cell proliferation and metastasis and inhibits apoptosis in human BC tissue. RNA sequencing analysis demonstrated that TUG1 expression is increased in HER2-enriched and basal-like subtypes in comparison to luminal A (139, 140). In contrast, evaluation of TUG1expression in BC tissues and cell lines showed that TUG1 was down-regulated and correlated with clinicopathological features(141). However, differences in the number of studied subtypes and number of samples can affect the overall results of TUG1 expression, although studies provide a novel biomarker in early diagnosis of BC in the clinic.

LncRNA H19

H19 is a multifunctional LncRNA, which plays an important role in growth control(109). H19 is increased in BC tissue compared with normal tissue and expression level of it is associated with proliferation, invasion, and metastasis (142). This LncRNA regulates gene expression in BC at epigenetic modification.

Lin Y and et al in a case-control study, found that elevation of H19 expression can increase the risk of breast carcinogenesis, moreover, the association was more significant in ER+, HER2-, and ER+HER2- molecular subtype of patients(143). Ye Fu and et al found that BaP treatment and H19 repression inhibits DNA-adducts formation. Also, they revealed evidence that H19 binding to S-adenosylhomocysteine hydrolase (SAHH) may inhibit methylation the LINE-1 in BaP-treated cells. Global methylation level has a negative correlation with BaP-induced DNA damage. In this research, they did not evaluate which type of DNMTs would be influenced during the interaction between H19 and SAHH in invitro, although another study had been revealed that H19 downregulation enhanced DNMT3B-mediated methylation(144). Aberrant H19 expression can be involved in diverse malignancies such as liver cancer(90), pancreatic cancer(91), gastric cancer(92), and tumorigenesis like breast cancer (94).

In addition to the above mentioned, epigenetic alteration-induced by the environmental factors in the promoter region of LncRNA may distinctly regulate their expression. Despite the suggestion of lncRNAs as novel diagnostic and prognostic tools it is important to note, that the aberrant expression of LncRNA is still controversial and can similar in a variety of cancers. Therefore, evaluation of LncRNA expression along with other epigenetic alterations in the susceptible genes can be useful as biomarkers for cancer risk prediction of environmental exposure such as PAH.

microRNAs

MicroRNAs (miRNAs) are noncoding RNA of ~22–23 nucleotides in length that regulate gene expression by pairing the mRNAs of protein-coding genes(92). The miRNA profile anomaly exists in every stage of BC, from beginning the tumorigenesis to metastasis. miRNAs act as tumor-suppressive or oncogenic that affect the cellular signaling pathways of proliferation, apoptosis, epithelial–mesenchymal transition (EMT), angiogenesis, growth, and metastasis. Also, miRNAs can provide valuable information about BC subtyping, treatment monitoring, diagnosis, and prognosis(91). A class of miRNAs that are known epi-miRs(145) can interact with epigenetic modification factors, such as DNA methylation and chromatin remodeling, to regulate gene expression in response to environmental stimuli. Because of the potential for one miRNA to target multiple gene transcripts, some epi-miRs perform

two functions in the breast. For instance, when the miR-22 targets HDA4, KAT5, TET1, TET2, and TET3, it acts as an oncomiR while by targeting the KDM7B and HDAC3, it affects as a tumor suppressor miRNA(112).

Environmental pollutants may change miRNA expression patterns(146) (96) (121, 147). BaP increased the expression of the miR-483-3p in the primary culture of rat hepatocytes while the AhR inhibitor decreased the level of the corresponding miRNA(148). Xiaoxi Huang et al showed that the expression level of miR-483-3p is significantly was reduced in BC cell lines(149) while other study reported that miR-483-3p expression was increased in BC cell lines(150) that their variety results can due to using different methods. miR-483-3p targets cyclin E1 and decreases MCF-7 cell proliferation(149). CyclinE1 forms a complex with CDK2 and then promotes entering the cell cycle from G1 to S phase(151). These results suggest that miR-483-3p may serve a role in tumorigenesis of BC.Other study demonstrated that the expression of miR-142-5p is downregulated after exposure to BaP (152) and negatively associated with plasma BPDE-albumin adducts(121). miR-142-5p downregulation is observed in luminal A/B BC subtypes while upregulation of miRN-142-5p is reported in HER2+ and Basal-like BC subtypes(91).miR-143-3p is linked to downregulate the genes expressed in cells that are treated by BaP. miR-143-3p upregulated diminishes proliferation and migration via modulating the MAPK7 in BC cell lines(153). Increase expression of miRNA-25 through PAH-activated AhR have a putative role in the regulation of tumor suppressor genes such as p53(154).

Hongyi zhang reported that miR-7 as a tumor suppressor could indirectly repress the STAT3 expression. They showed that miR-7 identified the 3'UTR of KMT1E and resulted in the suppression of STAT3. STAT3 downregulation leads to inhibition of c-myc, TWIST, and miR-9 expression in BCSC cell that subsequently decreases the EMT and metastasis. Moreover, they found that HOTAIR via PRC2 indirectly inhibits the miR-7expression. Due to PAH impact on HOTAIR regulation(155), it may be considered that PAH by targeting miRNA and LncRNA plays epigenetic alteration roles.

Some miRNAs bind directly to the 3'UTR of mRNA and subsequently, downregulate ESR1 expression (156-158). it is reported that miRNA-148a reduces DNMT1 expression that outcome is upregulation of ER expression(159). Some PAHs have estrogenic or antiestrogenic activities, because of their structural similarities. They can bind to both ERs, affecting their cascade signaling and increasing the risk of BC(160). Estrogen Receptorsα (ERα) and Estrogen Receptorsβ (ERβ) are two different isoforms of ERs that execute estrogen biological functions. They can trigger genomic and non-genomic signaling cascades. In the genomic pathway, ERs dimerize after binding with hormone and then bind to regulatory elements of target genes(161). During non-genomic signaling, they involve in the activation of the proteins. The ER expression dominantly is regulated by the epigenetic processes including, ER promoter hypermethylation and histone deacetylation. The invitro study demonstrated that ERα expression can be induced by epigallocatechin-3-gallate (EGCG) as a DNA methyl teransferase (DNMT) inhibitor as well as trichostatin A (TSA) as an HDAC inhibitor in ER-negative breast cancer cells(163). It is reported that BaP exposure may disrupt the expression of BRCA1 in ER+ MCF-7 cells (164). In this regard, the epigenetic components that regulate ER expression may present as a predictive factor for PAH- related breast cancer risk. In total, epidemiology studies need to reflect PAH-specific miRNA signatures that may act as epimiR, and miRNA biosynthesis. Involved enzyme in miRNA prossess may undergo dysregulation and leads to abnormal miRNA expression profiles(165).

CONCLUSION AND PERSPECTIVE

Personalized medicine makes decisions about drug treatment and disease management, based on some cancer genes harboring genetic changes. It predicts the patient response to specific anticancer drugs. Moreover, associated data, such as the levels of RNA, proteins and various metabolites. PAH epigenetically regulates some breast cancer-related genes, which contribute to the initiation of the pathogenesis of breast cancer. Epigenetic modification may do histone changes prior to the DNA methylation. Epigenetic modification, as biomarkers, can help to design preventive strategies and diagnose at-risk subgroups and premise of personalized medicine. Although studies have revealed that exposure to environmental pollutions may alter epigenetic modifications, many studies have used cigarette smoke, which includes a mixture of several substances or BaP alone. It has been shown that hypo- or hypermethylation at multiple promoter regions in breast tumors and LINE-1 hypomethylation in the blood of controls are associated with PAH source. PAH directly or indirectly by the production of activated metabolites can cause DNA adducts formation and subsequently genetic mutation. PAH may influence methyl modification through different pathways. PAHs change methylation modification by interacting with the AhR receptor and activating downstream genes. Another mechanism is the recruiting non-coding RNA, and subsequently, enzymes that are involved in the methylation process. Other mechanisms that involve in methylation is PAH-DNA adducts formation. Base on the number of methylated cytosines and its positions, DNA-adducts formation in CpG islands may induce hypo/hypermethylation in the promoter regions. Due to the low methyl groups in the promoter region of

suppressive genes, it seems that the formation of PAH-adducts and the created structure, may recruit the methylation compounds, which cause gene silencing. It is recommended that changing the length time of PAH-exposure, dose-response, diet, and the use of antioxidants may decrease the effects of PAH on epigenetic modification. Although the relationship between PAH-DNA adducts, PAH exposure, and BC susceptibility have been identified, there are still many unresolved questions about the effective mechanism of the PAH. Overall, a better understanding of epigenetic might open options for breast cancer prevention and follow up through the development of biomarkers reflecting exposures to PAH pollutants and predicting the risk of future breast cancer.

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http://pmjournal.ir Original Article

Spring 2022, Volume 7, Issue 25(15-24)

Molecular Detection of Fungal APR1 Gene in Serum of Multiple Sclerosis Patients: A Personalized Medicine Research

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DOI: 10.22034/pmj.2022.253551

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Submitted: 2022-02-18
Accepted: 2022-4-23

Keywords:
Personalized Medicine
Multiple Sclerosis
Detection of Fungal DNA
Candida albicans

Abstract:
Due to the lack of reliable biomarkers and a thorough understanding of the etiology of multiple sclerosis (MS), the treatment strategy in MS requires a personalized medicine framework that goes beyond the precision medicine idea. A patient-centered approach is necessary for personalized treatment, and the identification of pathophysiological processes should be employed to help classify diseases. Intracellular aspartic proteinase-A enzyme is expressed by the *APR1* gene and is one of the important factors in the development of systemic candidiasis caused by *Candida albicans*. The aim of this study was molecular detection of fungal DNA in serum of MS patients and to evaluate the expression of the *APR1* gene in *C. Albicans* isolates obtained from patients with multiple sclerosis (MS) and controls. The samples were obtained from 100 MS patients with candidiasis and 100 matched controls of healthy individuals during 2018 - 2019. The evaluation of *APR1* gene expression was performed using the reverse transcriptase-polymerase chain reaction (RT-PCR) method. There was a statistically significant difference in *APR1* gene expression of *C. Albicans* strains between MS patients (mean± SD: 0.5008 ± 0.09518) and the control group (mean± SD: 0.7513±0.10505) (P = 0.000). The mean values of EDSS were 1.4074 ± 0.0082 after antifungal treatment and 2.0519 ± 0.1123 before antifungal treatment (P = 0.000). Differences in active fungal infection between patients and controls indicate the importance and possible role of fungi in MS patients. The results suggested that *APR1* gene expression in *C. Albicans* strains isolated from MS patients may be an important factor for invasive *C. Albicans* strains in the progression of MS disease. Because fungal infections in the serum causes more activity of the body's immune and defense system and directly affect the activity of the immune system, it further destroys the central nervous system.

INTRODUCTION

To better meet the requirements of each patient, customized medicine often focuses on managing a patient's condition. Informed permission from the patient allows them to participate in the decision-making process, which is crucial to personalized medicine for physicians. According to the European viewpoint, illness taxonomy is influenced by the characterization of pathophysiological processes, with the health of each individual at the center of customized care. Realizing the objectives of customized medicine is made possible by integrating multidisciplinary involvement and research, data processing, infrastructure and resources, and collaborative decision-making (1). Due to the absence of reliable biomarkers and a thorough understanding of MS etiology, personalized medicine, as opposed to precision medicine as discovered in cancer, would be the preferred therapy strategy in

multiple sclerosis (MS). Multiple sclerosis (MS) is one of the inflammatory diseases of the central nervous system (CNS) demyelinating which mainly results in the neurological disability in the young population is increasing (1). The clinical manifestations of MS vary in patients and depend on the location of the injured nerves. The cause of MS is unknown but it is clear that environmental factors and hereditary factors are involved in determining the likelihood of getting MS (2). The broad spectrum of symptoms of MS considerably impacts the quality of life experienced by patients and their families to a greater extent than several other chronic diseases (3). Although etiological factors are not established, some data suggest that non-genetic (environmental) factors, especially infectious agents (e.g. fungal or bacterial infections), may play a role in MS (4). Numerous infectious agents are suspected of triggering MS, and emerging evidence suggest links between established MS and gut microbiota (5). Though most studies focus on bacteria, fungi may also play an important role (6). Many links between fungi and diseases involving idiopathic inflammation have been found recently. Among these, *Candida albicans* stands out as one of the most consistent risk factors (7). Nevertheless, some features of MS epidemiology are not explained and strongly suggest a key role of other infectious agents like *Candida albicans* (8). Antibodies against fungi are a risk factor for psoriasis (9), and fungicides improve symptoms (10). Antibodies against fungal mannoproteins are a risk factor for ankylosing spondylitis (21), systemic lupus erythematosus (11), sarcoidosis (12), and Crohn's disease (13). Few groups have considered the role of fungi in MS. In 1981, Truss reported the resolution of symptoms in five MS cases following antifungal therapy (14). In 2008, Ramos and colleagues reported finding serum antibodies against *Candida* in seven out of eight MS patients, while finding none in 10 healthy controls (17). In 2010, this association was replicated in a larger case-control study (18). Endo proteinases, carboxy peptidases, and amino peptidases are vacuolar proteases that have intracellular proteolytic activities in *Candida albicans*. Intracellular proteinase A is one of the most important enzymes and is produced by the *APR1* gene in *C. Albicans* (19). It is demonstrated that this enzyme has functions in the *Candida* genus, such as nutrition, help with penetration and invasion, infection of host tissues, and the suppression of the immune system. In response to several types of stress, a group of genes is activated in *C. Albicans*, where each type of stress is responded to by a particular set of genes (20). It is suggested that the increase of *APR1* gene expression will impact *C. Albicans* compatibility in the change of environmental conditions. Fungal infections can play a major role in the development of MS, and fungal infections in MS may be due to immune disorders (21). *Candida* species can have an unconstructive effect in the clinical course of MS even though they may not be the main etiology of the disease. *Candida* infection among MS patients has not been adequately studied. However, the possibility that MS is caused by an infective agent has been put forward (22).

MATERIALS AND METHODS

Patients and Controls

One hundred MS patients with fungal infections were included in this study. The samples were obtained from four subtypes of MS disease. Of 56 patients with the relapsing-remitting subtype (RR). A total of 44 patients belonged to the other MS subtypes, including: i) secondary progressive (SP), ii) primary progressive (PP) and iii) progressive-relapsing (PR) subtypes. The control samples and MS patients were similar in age and sex. The expanded disability status scale (EDSS), which ranges from 0 to 10, was defined as measuring impairment in half-point increments between a normal neurologic examination and death from MS. Samples came from Yazd's Shahid Sadoughi Hospital.

Molecular Detection of Fungal DNA in Serum

In this study, 100 cases of patients with MS and 100 samples of healthy individuals were collected. To extract DNA from serum-infected patients and control people, the proposed method was used in kit DNG - PLUS. In this method, we moved a 100 - µL sample of serum to a 1.5 mL tube and added 400 µl Lysis buffer to the sample, and then the sample was shaken for 30 - 20 seconds. After that 350 µl of isopropanol was added to the tube then inverted the tube 10 times for 10 minutes centrifuge at 12000 rpm. Then decanted the Supernatant and 1 mL of wash solution (alcohol 70 %) was added to the sample. After mixing the sample with the wash solution, inverted the tube 10 times and centrifuge at 12000 rpm. After discharge of the supernatant, the sediment in the bottom of the tube dried for 10- 50 min at 65 °C on a heater block device (Nasl Omid Pajoohesh, Iran). After drying the sediment 30-35 µl of Dionysed sterile distilled water was added to the tube and solved the sediment slowly with the finger and vortex. Then set the sample for 5 min at 65 ° C. We stored the extracted DNA for long-term use at -20 ° C. finally, the optimized test was done on all samples of patients and control with positive and negative control, and the results were examined on the electrophoresis gel (1.5 percent gel). The sequence of a dedicated primer pair for 18srRNA is based on the previous literature (22) that is presented in Table 2. PCR Thermo Cycler (Biorad, Germany) was programmed for 35 cycles.

PCR amplification was carried out according to pervious studies (15, 16) in a final volume of 20

Table 1. Clinical Data of MS Patients

Clinical Information	MS Patients
Total individuals with MS	100
Age at onset in years, Mean ±SD	23.4±4.6
Female/male ratio	1/2
Disease duration in years, Mean ±SD	2.5±1.8
EDSS	
≤ 3	82
3.5 - 6	14
> 6	4
different treatment	
used MS & antifungal drugs	39%
did not receive any MS and antifungal drugs	40%
received MS drugs without using any antifungal drugs	21%

Table 2. Sequences of Primers

Primer Name	sequences of primers	Product size PCR
APR1	F: 5'-TCCACCAATCTACAATGCCA-3 R: 5'-ATTTCAGCCAATGAGGATGG-3	300 base Pair
18srRNA	F: 5'-CGGGGAAACTCACCAG-3' R: 5'-AAGGGCATCACAGACC -3'	575 base Pair

µL containing: 10 µL PCR master mix (PCR buffer, MgCl2, dNTP, 0.2 units of Taq polymerase), 1µL reverse primer, 1µL forward primer, 1µL template DNA and 7µL distilled water (Amplicon, Denmark).

Expression of Fungal APR1 Gene in Multiple Sclerosis Patients

A source for gene expression was taken from 31 of the 100 blood samples taken from MS patients that were reported to have fungal infections. Out of 100 main MS patients, 39 instances utilized both MS and antifungal medications at the time of the sample, while 40 cases did not. Without utilizing any antifungal medications, all 21 of the remaining MS patients got MS medications. From hospital blood donors and volunteer students, 100 control samples were collected.

Candida albicans Isolation

The clinical and control isolates of *C. Albicans* obtained from individuals were cultured onto sabouraud dextrose agar (SDA) (Merck, Germany) at 37°C for 24 hours. For isolating the yeast cells, *Candida* colonies were harvested from SDA (Merck, Germany) media and placed into a 1 mL microfuge tube with 0.5 mL distilled water. For equaling the cell number in different samples of *Candida*, the OD of the cells was read at 550 nm. Subsequently, *Candida* cell suspensions were heated at 100°C for 10 minutes and centrifuged at 4500 × g at 4°C for 15 minutes. Finally, the supernatants were used in the reverse transcriptase-polymerase chain reaction (RT-PCR) process.

RT-PCR for APR1 gene

To evaluate the expression of the *APR1* gene, the RT-PCR reaction was performed in three steps: 1) RNA extraction, 2) RT reaction and 3) PCR reaction.

RNA extraction

Total RNA was extracted using RNX plus buffer (Cinagen, Iran). Briefly, about 2×10^6 fungal cells were transferred to 1mL of RNX-plus buffer (Cinagen, Iran) in an RNase-free Microtube, mixed thoroughly, and left at room temperature for 5 minutes. A volume of 200 µL of chloroform (Merck, Germany) was added to the slurry and was mixed gently. The mixture was centrifuged at 13200×g at 4°C for 15 minutes; the supernatant was transferred to a new tube and was precipitated with an equal volume of isopropanol (Merck, Germany) for 15 minutes on ice. The RNA pellet was washed using 75% ethanol (Merck, Germany), briefly dried, and Resuspended in 15 µL of RNase-free water. The purified total RNA was quantified by a Thermo Scientific NanoDrop ONE (Thermo, USA).

RT reaction

A sample of RNA was used for first-strand cDNA synthesis, using 100 pmol oligo-dT (Takapoo zist, Iran), 15 pmol dNTPs (Takapoo zist, Iran), a 20 U RNase inhibitor (Takapoo zist, Iran), and a 200 U M-Mulv reverse transcriptase (Takapoo zist, Iran) in a 0.02 mL final volume. Twenty µL of solution were obtained and maintained at room temperature for 10

minutes. These solutions were incubated at 42°C for 60 minutes in a heat block (Nasl Omid Pajoohesh, Iran) and then at 70°C for 10 minutes.

PCR reaction

Two-pair primers (SinaColon, Iran) including *APR1* as the proteinase-A gene, and 18SrRNA as the housekeeping gene were selected for this study [See table 1] (21). PCR amplification was carried out in a final volume of 20 µL containing: a 10 µL PCR master mix (PCR buffer, MgCl2, dNTP, 0.2 units of Taq polymerase), 1µL reverse primer, 1µL forward primer, 1µL template cDNA and 7µL distilled water (Amplicon, Denmark). The PCR gradient showed that the best annealing temperature was considered 55.5°C for both *APR1* and 18SrRNA genes.

Agarose Gel Electrophoresis

To show the cDNA bands of *APR1* and 18SrRNA genes, the PCR products were run on 1.5% (w/v) agarose gel (Merck, Germany). A 1kb DNA ladder (Bioflux, USA) was used as a marker at this stage.

Statistical analysis

The statistical analysis of the expression of the *APR1* gene in *C. albicans* strains isolated from MS patients and from controls was performed using the independent T-test (SPSS, version 16) method. The differences were considered to be significant at $P < 0.05$. The correlation between *APR1* gene expression with age at onset in years and EDSS was investigated using the Pearson correlation test, and the correlation between the *APR1* gene with MS Patients and sex was detected using the Chi-square (X2) test in this software program.

RESULTS

Prevalence of fungal infections in patients' serum

The data analysis of sex, age, disease duration, EDSS, and different treatments of MS are shown in Table 2. A total of 100 MS patients and 100 healthy controls participated in this study; 68 of the formers were male, and 32 were female. MS was observed in patients ranging from 16 to 40 years (mean age: 23.4 ± 4.6 years). The mean disease duration ranged from 2 to 10 years (mean duration: 2.5±1.8 years). The mean EDSS ranged from 0.5 to 7 (mean value: 2.25 ± 1.5), and in most of the patients, the EDSS was less than 3 (82%). But most patients (40%) did not take any MS and antifungal drugs, indicating an unequal drug distribution system In Iran. Among these patients, 31 cases had serum fungal infection. The PCR gradient showed that the best annealing temperature was considered 55.5°C for 18SrRNA gene. The detection limit of the PCR assay was determined by 40 copy of fungal DNAs using serial dilutions where the number of genomes in each was determined (Fig. 1).

Human DNA, mouse, herpes simplex virus 1, herpes

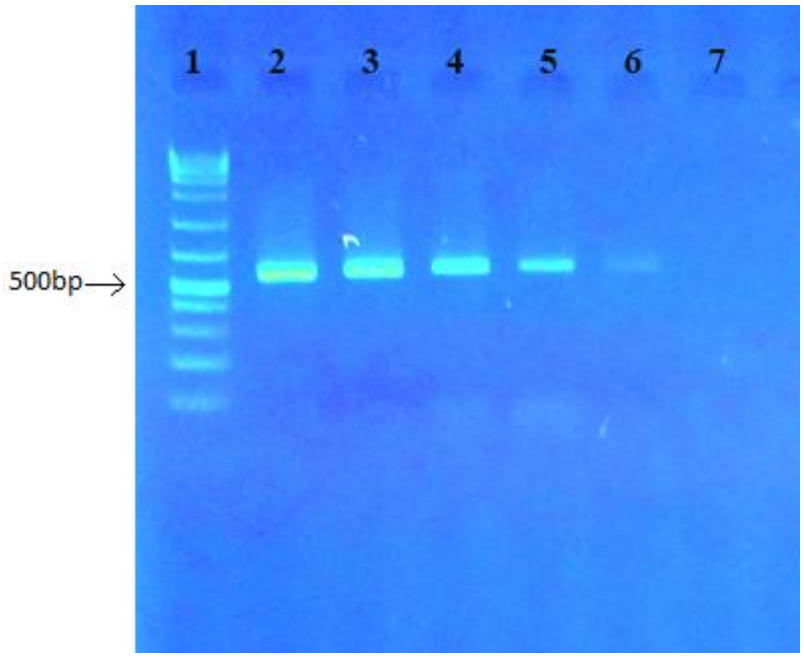

Fig1. Optimization of LOD (Optimized by fungal DNA)
1. Marker size: 100bp DNA Ladder 2. Fungal product size, 575 bp
3. The number of 40000 fungal DNA in a PCR reaction
4. The number of 4000 fungal DNA in a PCR reaction
5. The number of 400 fungal DNA in a PCR reaction
6. The number of 40 fungal DNA in a PCR reaction
7. The number of 4 fungal DNA in a PCR reaction

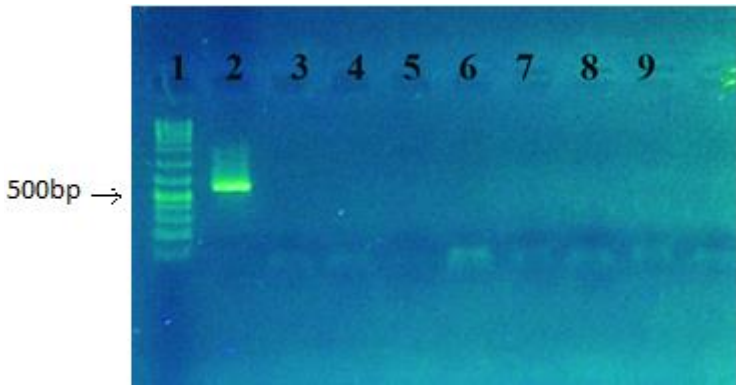

Fig2. Specificity of PCR test for fungal identification.
1. Marker size: 100bp DNA Ladder 2. Positive control sample (Fungal product 575 bp)
3. Mouse DNA 4. Herpes simplex virus1 5. Herpes simplex virus2
6. Hepatitis B Virus DNA 7. Adenovirus DNA 8. Staphylococcus aureus DNA
9. Negative Control

simplex virus 2, hepatitis B virus, adenovirus and Saccharomyces Cerevisiae were used to determine the specificity of the PCR test with positive and negative control samples (Fig. 2).

Fisher test was used to determine the significant relationship between fungal DNA presence in patients and controls. Then, the presence of the 18srRNA gene was analyzed by molecular PCR test and significance level for this difference was determined by Fisher test 0.052 and the results were writing in table 3.

Table 4 shows that 19 individuals had never taken any medicine, 11 had only taken MS medications, and 1 had both MS and antifungal medications. This shows how susceptible MS patients are to fungus infections and how directly antifungal medications affect these patients' therapy.

As shown in Table 5, the correlations between *APR1* gene expression and clinical variables were investigated. There were statistically significant correlations between *APR1* gene expression and age at disease onset as well as EDSS (P = 0.000), whereas no significant correlation was found between the gene expression and MS patients well as sex (P > 0.05). Because of some limitations in doing laboratory examinations, among the clinical features of MS patients, only the EDSS of the MS patients was

Table 3. Fisher's exact test results

Group	Frequency/ percent	Fungal DNA in serum	No fungal DNA in serum	Total
MS Patient	Frequency	31	69	100
	Percent	31%	69%	100%
Control	Frequency	4	96	100
	Percent	4%	96%	100%

Table 4. Distribution of drug use in MS patients with fungal infection in serum

different treatment	Case of patient	
did not receive any drugs	19	61.2%
received MS drugs without using any antifungal drugs	11	435.48%
MS & antifungal drugs	1	3.22%

Table 5. Relationship between APR1 Gene Expression and Age, Sex and EDSS

Clinical Variables	APR1 Gene Expression, Mean± SD	Correlation, P Value
Sex		0.38[2]
Male	0.5227±0.11149	---
Female	0.5200±0.11733	---
Age at onset in years		0.000
EDSS		
≤ 3	0.5223±0.06414	
3.5 - 6	0.4431±0.12960	
> 6	0.1527±0.02903	

Table 6. Mean Ratio of APR1/18SrRNA in MS Patients and Controls

MS patients, No.31	0.5008 ± 0.09518
Controls, No.4	0.7513±0.10505
P value	0.000

investigated after 9 months. Of the treatment of these patients with antifungal drugs and the level of fungal DNA in serum and consequently fungal infection decreased but there was no significant relationship between fungal infection and incidence of MS. But this demonstrated that the progression of MS decreased after the treatment of patients with antifungal drugs. The results showed that the mean values of EDSS were 1.4074 ± 0.0082 after antifungal treatment and 2.0519 ± 0.1123 before antifungal treatment, representing a statistically significant difference between what occurred before and after treatment with antifungal drugs (P=0.000). Our findings demonstrated that the progression of MS decreased after the treatment of patients with antifungal drugs.

In this study, *APR1* gene expression was investigated in *C. Albicans* strains isolated from 31 MS patients (Table 6). According to the data, there were no statistically significant differences in the mean ratio of *APR1* to 18SrRNA genes in *C. Albicans* strains isolated from MS patients with candidiasis. The number of people susceptible to fungal infection was significantly higher in the MS group (n=31) than in the control group (n=4), which is a significant difference. That is, people with MS are susceptible to fungal contamination and further destruction, indicating the need for antifungal drugs in these individuals. In contrast, the mean ratio in *C. Albicans* strains isolated from 31 of MS patients (0.5008 ± 0.09518 unit/mg) was significantly less than that of the 4-control group (0.7513± 0.10505 unit/mg) (P = 0.000). The results of *APR1* and 18SrRNA gene expressions showed different expressions among MS patients and controls. As observed, the *APR1* and 18SrRNA in control subjects, it is more expressed than in MS patients. This may be due to immune system involvement in MS patients, the immune system is involved with monocytes, macrophages, and cytokines. High immune activity may reduce the existing fungal infection in healthy individuals.

The comparison of *APR1* gene expression showed that the gene expression increased in *C. Albicans* strains obtained from controls when compared to MS patients. This concept means that fungal contamination cannot cause MS in humans but as a risk factor can lead to the destruction and weakening of MS patients. Because fungal infections in the serum causes more activity of the body's immune and defense system and directly affect the activity of the immune system, it further destroys the central nervous system.

DISCUSSION

Varied molecular and epidemiological evidence supports the role of infections in MS. Fungal infection is the most strongly associated infection, though the underlying mechanisms are not firmly established. Several *Candida* species are widely distributed throughout the human population as both commensal organisms and as intermittent pathogens (22). In the clinical arena, blood cultures are the main assay for routine detection of candidiasis (23). However, microbiological confirmation is difficult because blood cultures can be negative in up to 50% of autopsy-proven cases of deep-seated candidiasis or may only become positive late in the infection (23). The aim of this study was molecular detection of fungal DNA in serum of MS patients and to evaluate the expression of the *APR1* gene in *C. Albicans* isolates obtained from patients with multiple sclerosis (MS) and controls. In this study, we investigated the molecular characterization of the fungal DNA in serum patients with MS and healthy individuals in the PCR method. Our idea was to investigate the role of fungal infection in MS disease. So we collected 100 samples of serum with MS and 100 healthy control samples. The results of the Fisher's test showed that 31% of the samples of MS patients had a fungal infection, whereas that of healthy individuals was only 4%. This difference is quite significant and indicates the possible effect of fungal infections on MS. Rasmos et al. In 2008 investigated the presence of anti-*Candida Albicans* antibody and the parasitic, Famata, Glabrata, and Crocus strains in serum of MS patients using Western blot and immunofluorescence and slot-blot analysis. The results of the slot-blot technique indicate the presence of different pathogenic species in patients (except one) as opposed to controls. Following DNA extraction from the full blood of the patients, they studied the Molecular Study of the presence of the fungal DNA using PCR. The fungal DNA was present in the environmental blood of the MS patients against the control. In addition to serum, CSF of patients and controls was evaluated for the presence of specific antigens against different fungal species using the slot blot technique, Results showed antibodies against *Candida* Famata in CSF of patients, unlike controls (24). Another interesting study was conducted by Yoshitomi et al. In 2005. They observed that injecting a fungal-purified beta-glucan antigen into SKG mice genetically susceptible to rheumatoid arthritis caused the disease. Beta-glucan injection into mice causes the activation of the dendritic receptor 1 dendritic TBs and initiates innate immune responses

and eventually rheumatoid arthritis (25).

In addition, Pisa and colleagues reported in 2011, a fungal infection of *Candida* Famata in a patient with MS. The findings of slot blot, PCR, and immunofluorescence all suggest this fungal infection in this patient and the necessity of investigating more types of fungal infections in MS patients to prescribe appropriate medications (26). Other studies have been made about the possible relevance of fungal infections and other nervous system diseases such as Alzheimer›s and Parkinson›s disease. Proteomic analysis of the brain of Alzheimer›s patients indicates the presence of fungal proteins. The presence of the fungal DNA in the brain of patients with Alzheimer›s was investigated using the PCR method and the presence of the fungal DNA in the brain of Alzheimer›s patients was found in comparison with the control. Interestingly, the Aβ peptide has been shown to have antimicrobial activity against Candida albicans. This finding could be a good guide to the physiological function of this peptide. By proving the presence of fungal proteins in the brain of Alzheimer›s patients, it can be said that fungal agents have entered the brain of patients. Of course, the number and type of fungal species vary in different patients. Considering the presence of fungal in the brain, it seems that the peptide is synthesized to perform its antifungal activity. Continuous synthesis of this peptide results in the accumulation of amyloid peptides Therefore, fungal infection induces accumulation of Aβ peptides which in turn accumulates tau protein and neuronal filamentous waste and ultimately neurodegeneration; it also stimulates the secretion of inflammatory cytokines. Some patients with Alzheimer's disease have Cryptococcus infection, however, after the antifungal treatment, their clinical symptoms are greatly reduced. Another interesting point is that there is inflammation and vascular dystrophy in many Alzheimer's patients, which is consistent with the finding that fungal agents cause vascular inflammatory reactions (26). In addition to Alzheimer's disease, the association of fungal infections with Parkinson's disease has also been studied. Patients with Parkinson's also suffer from motor problems, such as problems with facial muscle movements, in addition to motor symptoms. These immobility problems usually occur in patients with salivary dermatitis (Seborrheic dermatitis) and increased sebum secretions, which is due to overactive parenchyma and systemic effects of melanocyte-stimulating hormone (MSH). Treatment of Parkinson's patients with the combination of L-dopa also reduces sebum secretions. According to 2016 research Trusted Source, both MS and PD can affect a person's physical and cognitive functioning. These conditions typically have more severe physical effects than cognitive effects, particularly during the early stages of the diseases. Some of the symptoms of MS and PD are similar (26). The yeast in Malaysia needs certain internal lipids for growth. Yeast Malazia increases androgen secretion by increasing sebum secretion in patients infected with this fungus, triglycerides, and cholesterol are usually high, but the levels of squalene and free fatty acids are significantly lower than in healthy individuals. The free fatty acid is formed from triglycerides through the function of bacterial lipases such as propidium acne. These findings indicate that the imbalance between microbial flora leads to changes in skin lipid composition and provides conditions for the pathogenesis of other infectious agents. The pathogenesis of these factors is mediated by lipase and phosphatase since it initiates inflammatory responses through the release of arachidonic acid from sebum lipids. The general characteristics of all these diseases are similar to MS and similar mechanisms are involved in their occurrence. Fatty acids have direct effects on keratinocytes. The arachidonic acids metabolized in the cyclooxygenase pathway, in turn, are a source of proinflammatory eicosanoids that cause inflammation and damage to the stratum corneum. The globsa is the most pathogenic species of this genus and has more lipase and phosphatase enzymatic activity. This yeast appears to be involved in allergic reactions through other mechanisms as well. Some of these yeast enzymes, such as proteases, glycosidases such as beta-glucuronidase, and leucine arylmidaz, are considered antigens and produce atopic immune responses (27). To the best of our knowledge, there is not enough information about *APR1* gene expression in *C. Albicans* isolated from MS patients, so this study can be considered a novel research study. Previous studies indicated the effect of environmental factors (non-genetic), especially infectious agents, on MS (28). There is a hypothesis about the association between some pathogenic yeasts, such as *C. Albicans C. famata*, *C. parapsilosis, C. glabrata*, and MS (29). In MS patients with candidiasis, demyelinated lesions have been shown in the central nervous system (30). Intracellular proteinase A is one of the most important enzymes in *C. Albicans* that is expressed by the *APR1* gene. This enzyme has an important role in cell survival under stress conditions in *C. Albicans*, and the amount of this enzyme increases in the first few hours of nitrogen starvation when compared to mycelium growth (31). The present study showed that the expression of the *APR1* gene in controls was more than that of MS patients. This difference could be related to both the situation of yeast cells in the human body and the existence of immune disorders. The immune system is involved in infectious diseases, such as candidiasis, and also in MS. In *Candida* infection, monocytes/macrophages synthesize chemokines and cytokines, leading to increased immune responses

(32). There are serious immune or metabolic deficiencies in patients with opportunistic fungal infections and these patients are also under immunosuppressive therapy (33). The present study showed that all MS patients were recently involved in *Candida* infection and some of them did not use any antifungal drugs. Some of these patients were treated with MS drugs and have immune dysfunction. Of the treatment of these patients with antifungal drugs and the level of fungal DNA in serum and consequently fungal infection decreased but there was no significant relationship between fungal infection and incidence of MS. But this demonstrated that the progression of MS decreased after the treatment of patients with antifungal drugs. The results showed that the mean values of EDSS were 1.4074 ± 0.0082 after antifungal treatment and 2.0519 ± 0.1123 before antifungal treatment, representing a statistically significant difference between what occurred before and after treatment with antifungal drugs (P = 0.000). Our findings demonstrated that the progression of MS decreased after the treatment of patients with antifungal drugs. The number of people susceptible to fungal infection was significantly higher in the MS group (n=31) than in the control group (n=4), which is a significant difference. That is, people with MS are susceptible to fungal contamination and further destruction, indicating the need for antifungal drugs in these individuals. In contrast, the mean ratio in *C. Albicans* strains isolated from 31 of MS patients (0.5008 ± 0.09518 unit/mg) was significantly less than that of the 4 control group (0.7513± 0.10505 unit/mg) (P = 0.000). It was detected that there are correlations between *Candida* infection and the increase in MS progression. Because fungal infections in the serum causes more activity of the body's immune and defense system and directly affect the activity of the immune system, it further destroys the central nervous system. Regarding the significant role of proteinase-A in the production of new proteins in *C. Albicans*, the *APR1* gene was found to be an important gene in the adaptation of this yeast in different situations. It was also found that there is a correlation between MS patients with fungal infections and the etiology of MS with fungal agents (34). In conclusion, the Fisher test was used to determine the significant relationship between fungal DNA presence in patients and controls showed that the MS patients people had fungal infections in serum more than control people. In addition, the expression of the *APR1* gene in *C. Albicans* was higher in controls than in MS patients using the RT-PCR technique. This study finds that the over-expression of the *APR1* gene can be an important factor in the development of candidiasis caused by *C. Albicans*. In addition, the results here will be useful precursors for achieving the future goal of further research of intracellular proteinases in *Candida* species because of their importance in human pathogenesis. The results of this study propose that *Candida* infection may be associated with the increased progression of MS. Therefore, it is suggested that future studies should make clear how *Candida* species act in the pathogenesis of MS. The comparison of *APR1* gene expression showed that the gene expression increased in *C. Albicans* strains obtained from controls when compared to MS patients. Because fungal infections in the serum causes more activity of the body's immune and defense system and directly affect the activity of the immune system, it further destroys the central nervous system. Given the role of fungal agents in autoimmune diseases such as MS, further studies are suggested. Fungal infection can be investigated using other molecular methods, along with serological and culture methods. It is also recommended that other Target genes be examined as well as the presence of DNA, antigen and fungal antibodies in the cerebrospinal fluid of patients.

Acknowledgements

The authors would like to thank the staff members of Biotechnology Research Center of Islamic Azad University, Shahrekord Branch, Iran for their help and suppor

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http://pmjournal.ir Review Article

Spring 2022, Volume 7, Issue25(25-29)

The Personalized Medicine Approaches in The Treatment of Corona Virus Disease2019- (COVID19-): A Review

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DOI: 10.22034/pmj.2022.253552

Submitted: 2022-01-28
Accepted: 2022-05-23

Keywords:
Personalized Medicine
Covid-19
Corona virus
Angiotensin-converting enzyme 2

Abstract:
Personalized medicine is the clinical treatment of diseases that is tailored to the physiologic, molecular genetics and lifestyle characteristics of the patient.
Personalized medicine can be considered as a new approach to face diseases and develop traditional methods for their diagnosis and treatment. This novel field of medicine has the potential of changing identification and management of health problems strategies. Corona virus disease 2019 (COVID-19) is an infectious disease that affects the lungs of patients. This novel outbreak was first reported on 31 December 2019 in Wuhan, the capital of Hubei province of China, and it had many effects on people's lives all over the world in various economic, social and health fields until now. Since the start of the pandemic Covid-19, the World Health Organization (WHO) has expressed concern about the public health emergency. Although the disease has mild symptoms and similar to a common cold in most people, in some cases it can lead to pneumonia, acute respiratory distress syndrome, multi-organ dysfunction, and even death. Therefore, due to the different effects of this disease in individuals and even families, the role of personalized medicine becomes more significant and sensitive. Considering the rapid spread and global crisis of Covid-19, recent research has focused more on the control and treatment of the virus. The main goal of this paper is the investigation of different effects of the virus on patients and study of the personalized medicine roles in the control and treatment of the disease.

INTRODUCTION

Corona virus 2: Pathogenicity, Transmission and Spread

After a severe acute respiratory syndrome disease broke out in Wuhan, China in December 2019 and it affected the whole world; on 7 January 2020 the virus was identified as a corona virus with more than 95% similarity to bat corona virus and more than 70% similarity to SARS-CoV. The body's areas affected by COVID-19 were six times higher than those affected by severe acute respiratory syndrome (SARS) (1).

Corona viruses are classified into four main genera: α-CoV, ß-CoV, γ-CoV and δ-CoV. The α-CoV and ß-CoV are capable of infecting mammals, while γ-CoV and δ-CoV tend to infect birds(2). Already, six corona viruses were known to cause infection in humans. Among them, α-CoV strains (HCoV-229E and HCoV-NL63) and ß-CoV strains (HCoV-HKU1 and HCoVOC43) have low infectivity and cause only mild respiratory infections similar to common colds. The two most well-known ß-CoVs; SARS-CoV and MERS-CoV; can cause severe and possibly life-threatening respiratory symptoms. Based on available evidence from genome sequencing and evolutionary analysis, bats have been proposed as the reservoir and natural origin of SARS-CoV-2. Bats may transmit the virus to humans through unknown intermediate hosts (3-5). Corona virus can easily move between species. In this way, Lu et al found that an important factor in corona transmission is the spike glycoprotein S1, which tightly binds to the angiotensin-converting enzyme2 (ACE2) receptor and enters the host cell. The basis of the corona virus pathway depends on ACE2 and protease/serine subfamily 2 (TMPRSS2) cleavages, particularly in the airways and cavities (6).In an experiment by Yang et al., mice over expressing human ACE developed more severe disease when infected with SARS-COV (7). ACE2 is found in various tissues

and organs such as kidneys, heart, intestines and blood vessels (8-15).

Also, ACE2 is expressed in type 2 epithelial cells, which play a key role in pulmonary gas exchange by producing surfactant. Therefore, any damage to these structures causes a disorder in the lungs (16).SARS-COV down regulates ACE2 expression, thereby causing damage to the lungs. Hence, ACE2 plays a dual role in both SARS-COV entry into cells and lung protection against injury (17-19).

The rate of human-to-human transmission of the new corona virus is significantly high, which leads to a wide expansion of clinical observations in infected patients (20). Although the absolute number of deaths related to COVID-19 was high, it appears that SARS-CoV-2 has a lower mortality rate than SARS-CoV or Middle East respiratory syndrome corona virus (MERS) (21). There is a lot of evidence that many cases of COVID-19 are asymptomatic, but they can transmit the virus to others (22). Detection of asymptomatic infections is essential for the prevention and timely control of COVID-19 worldwide (23). The spread of COVID-19 has been rapid. Due to the high binding affinity of SARS-CoV-2 to human angiotensin-converting enzyme 2 (ACE2), the main cellular receptor of the corona virus, its transmissibility is much higher than that of SARS (24). In the early stages of infection, the effective number of replications of SARS-CoV-2 was estimated to be 2.9, compared to 1.77 for SARS virus. Direct transmissions, airborne and contact are the three main transmission routes of SARS-CoV-2 (25). Many patients with COVID-19 have flu-like symptoms, including cough, fever, fatigue, anorexia, sputum production, and shortness of breath (26). Most people experience only mild symptoms, However, COVID-19 can also cause severe acute respiratory syndrome (ARDS), which often leads to intensive care unit (ICU) admission and death (27). Respiratory failure caused by ARDS is the main cause of death; but due to viral invasion of organs with high ACE2 expressing cells, patients with non-respiratory symptoms such as kidney failure or damage to the function of male gonads have also been reported in some cases (28,29). Based on these reports, the duration of the infection period of COVID-19 appears to consist of three basic phases: the first incubation period is largely asymptomatic, followed by the onset of symptoms in the second phase, which is nonspecific and non severe, with a subset of Patients progressing to the third stage with severe lung disease, it is often associated with extra pulmonary organs dysfunction (30-33).

Evidence-based medicine, personalized medicine and covid-19

The prediction of drug response of patients to make sure about patient safety and better drug effectiveness introduced “evidence-based medicine” in the early of 1950s and this was followed by the creation of personalized medicine field. Combination of modern medicine and molecular biology for treatment of patients separately based on individual characteristics in order to improve the treatment efficacy and better responses of patients to drug and reduce the drugs side effect is the main goal of personalized medicine. The evolution of genotyping techniques, biochips and single nucleotide polymorphisms (SNPs) in the past years has allowed researchers to find genetic differences between people in certain regions of genes and to perform treatment based on individual characteristics (34).

Researchers have proven that variations of human genome increase the risk of diseases such as cancer, cardiovascular and neurodegenerative diseases, diabetes and infectious diseases. However, environmental and lifestyle factors also have a great impact on disease progression, so personalized medicine should consider genome variations along with environmental factors in order to provide more efficient solutions for treatment (35).

Evidence-based medicine requires patients who share enough of a common condition that their responses are generalizable to a clinical practice. This evidence-based medicine seems to be in contrast to personalized medicine, which emphasizes aspects of each patient and thus makes them unique and personal (36).

Despite all the worldwide achievement and efforts for diagnosis and treatment of Covid-19, a definitive solution for the treatment of this disease has not yet been provided by modern medicine. Patients with COVID-19 not only are not all the same, they can also differ profoundly in terms of severity and pathophysiology. The main factor of drug efficacy and toxicity in the covid-19 patients is the genetic background of them (37). recent studies showed that two ACE2 alleles (i.e., rs73635825 and rs143936283) probably reduced viral attachment and increase resistance to infection (38). Moreover, researchers demonstrated that chromosome 3p21.31 (rs11385942) and chromosome 9q34.2 (rs657152) related to the ABO blood group involved in

COVID-19 patients with respiratory failure (38). Other genomic variations associated with COVID-19 disease severity in chromosomes 1 (1q22.1), 2 (2p21.1), 3 (3p21.1–3), 6 (6p21.1), 8 (8q24.13), 9 (9q34.1–2), 12 (12q24.1–2), 17 (17q21.3), 19 (19p13.1–3) and 21 (21q21–q22 have been reported (39, 40). However, it is still difficult to ensure about this findings because the limitation of testing methods and study population.

Along with the pathophysiology of the infection caused by COVID-19, the immune responses in these patients can be categorized into phases. Phase I, the initial innate immune response that is critical for the host to provide an antiviral defense in the lung, and

the subsequent phase that leads to intense local and systemic immunity are responses that contribute to morbidity and mortality (36). The heterogeneity of COVID-19 requires us to apply the principles of both evidence-based medicine and personalized medicine.

Due to the limitations of experimental methods for identification of covid-19 pathogenicity, various drugs such as IFN-a, lopinavir/ritonavir, chloroquine phosphate (or hydroxychloroquine), azithromycin, ribavirin, arbidol and remdesevir are recommended for patients at different stages of the disease. According to personalized medicine principles, the effectiveness of these drugs will definitely be different in diverse regions of the world. Unfortunately, there is no global organization to monitor the impact of environmental and regional factors, weather, genetic factors and lifestyle on the effectiveness of covid-19 drugs (34).

Personalized medicine approaches to COVID-19, including the pathogenesis of COVID-19 and the development and use of diagnostic kits, have played a key role in countries' response to the outbreak (32, 33). The medicine approaches are moving from traditional medicine to personalized medicine. As the law develops and improves, we are more able to employ the principles of personalized medicine. Personalized medicine strategies in other infectious diseases also show unique advantages. Considering these issues, we believe that with rapid development of molecular diagnosis technology and increasing awareness of the cost-effectiveness of personalized medicine, the applications of personalized medicine in the field of infectious diseases will lead to better management and treatment of them (41, 42).

Researchers have discovered hundreds of genes with mutations that contribute to human disease, identified genetic variation in patients' responses to dozens of treatments, and are working to target the molecular causes of some diseases. In addition, scientists are developing the use of diagnostic tests based on genetics or other molecular mechanisms to better predict patients' response to targeted therapy. Currently, personalized medicine is focused on the best ways to develop new treatments and optimize prescribing by guiding patients to the right drug, the right dose, at the right time. Achieving these goals includes scientific challenges, such as which genetic markers have the most clinical significance, limiting the off-target effects of gene-based therapies, and conducting clinical studies to identify genetic variation that is associated with drug response. As genetic researchers generate vast amounts of new information, the FDA (Food and Drug Administration) is developing regulatory science standards and the evidence required for the use of genetic information in drug and device development and clinical decision-making (43). On the other hand, genetic tests are not perfect and do not fully predict outcomes, partly because of most gene mutations. So it is necessary for physicians to understand the specificity and sensitivity of new diagnoses. It is also anticipated that some previously rejected drugs will be found to be safe and effective and will be approved for subgroups of patients with specific genetic markers (44).

CONCLUSION

Personalized medicine means escaping the "one medicine fits all" approach to medicine focusing on the individual characteristics of each patient. The transition from the old approach, traditional medicine, to the new approach, personalized medicine, requires the amendment of existing laws. Following the spread of the pandemic virus of COVID-19 and the infection caused by this virus and the different immune responses of people to this virus and the drugs prescribed for it, the role of personalized medicine became more prominent. In order to apply personalized medicine methods in COVID-19 healthcare, we must adhere to ethical and practical principles to ensure its safety and effectiveness.

At the same time, personalized medicine may be associated with challenges from different aspects. One of these challenges is people's awareness about personalized medicine. Patients should be empowered to participate in the decision-making process. Another challenge is funding for personalized medical research and diagnostics. Therefore, personalized medicine is very suitable for dealing with infectious diseases, including COVID-19. This method can be used to evaluate the pharmacogenetic characteristics of patients to help with treatment decisions and reduce the risk of side effects. Due to recent advances, personalized medicine can be more convenient, less expensive and more efficient in treatment.

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Spring 2022, Volume 7, Issue 25(30-35)

Evaluation of The Effects of Diazinon Toxin on Some Reproductive Parameters In Male Rats

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DOI: 10.22034/pmj.2022.253550

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Submitted: 2022-02-03
Accepted: 2022-05-18

Keywords:
Organophosphates
Diazinon
Sperm parameters
Oxidative stress

Abstract:

The reproductive system is affected negatively by the organophosphate insecticide diazinon (DZN). Numerous adverse effects on the reproductive system are brought on by it, including testicles degeneration, sex hormone disruption, decreased spermatogenesis, poor sperm quality, and fertility issues. The goal of the current study was to look at how diazinon affected the sperm parameter, sperm viability, and levels of sex hormones in adult male rats. The mature male rat was divided into five groups for this experiment: control (did not receive any substance), Placebo group (only 0.9 percent saline solution was consumed), and the other three groups received DZN (diazinon was administered at doses of 5, 10, and 20 mg / kg for 30 days). Within 30 days after the most recent doses, animals were killed. Radioimmunoassay was used to evaluate the amounts of serum testosterone, LH, and FSH. Sperm parameters such as motility and count were measured by CASA system. Sperm viability was also calculated by eosin-nigrosin staining. Following the injection of DZN, a substantial decrease in testicular weight and sperm concentration was seen. Additionally, DZN caused a significant decrease in serum testosterone concentration as well as sperm viability. Comparing DZN groups to the control and sham groups, LH and FSH levels were higher in the DZN groups. DZN is harmful to the reproductive organs and spermatogenic cells of mammals. Application of DZN should indeed be restricted to a specific framework.

INTRODUCTION

In contrast to the traditional method of treating everyone the same, personalized medicine involves applying specific medical procedures, medications, and/or processes to each patient to prevent, diagnose, and/or treat disease (1). This method is based on detailed knowledge of the individual's specific and explicit characteristics as well as the disease, including genotype, physiology, exposure to environmental, and lifestyle levels, among other factors (2). It is a ground-breaking strategy that incorporates variance into all facets of health care for the prevention and treatment of disease (2). To this end, it is crucial to understand the precise cause of disease and the underlying physiology when attempting to design treatment tools. From this approach, it is anticipated that the treatments will be more effective, cost-effective, and have fewer side effects, which will be beneficial for all patients (3).

Pesticide use in agriculture for crop conservation and pest control has been associated with difficulties with people's health and environmental degradation worldwide (4). Numerous studies have shown that the quality of human sperm has declined over the past few decades, and many have connected this to environmental exposures to pesticides at work (5,6). Although published research have suggested a link between pesticide exposure and male fertility, experts disagree over the precise detrimental effects of pesticides on male reproductive health (7). As a result, the impact of pesticides on human reproductive system is still debatable, and a rising number of researchs have concentrated on the diverse group of pesticides.

Agricultural workers' exposure to organophosphorus pesticides (OP) is a major health concern currently (8). They are extremely poisonous to insects and animals while being quickly digested (8). To control flies, lice, and other insect pests on ornamental plants and food crops, diazinon (DZN), an organophosphorate pesticide (OP), is used extensively in agricultural practices across the world (9). Concerns regarding DZN's impact on humans and laboratory animals have been growing. It prevents the activity of acetylcholinesterase and

other organic processes (10). DZN has been shown in several research to alter male rat blood factors, plasma testosterone, and glucose levels (11). However, little is known about how hazardous DZN is and how it affects male reproductive function. DZN has the potential to interfere with animal reproductive function, according to several research (12). The adverse effects of DZN on human reproductive function, however, have been well-documented. These include decreased libido after percutaneous treatment and notably lower levels of androgenic hormone. We hypothesised in the current investigation that DZN has a harmful impact on sperm parameters and hormone level that might be a risk factor for infertility since the detrimental effect of DZN on male reproductive function is still debatable.

MATERIALS AND METHODS

Animals and material

40 adult male rats weighing between 180 and 200 grams were purchased from the Pastor Institute. The rats were kept in solitary confinement in a room with a 12:12 light/dark cycle and an ambient temperature of 20°C. International Agricultural Chemistry gave diazinon.

Experimental groups

Adult rats were randomly divided into 5 groups, comprising: The control group (n=5) did not receive any substance. Placebo group: Only 0.9% saline solution was ingested. Experimental group 1: received diazinon toxin at a dose of 5 mg / kg orally for 30 days. Experimental group 2 received diazinon toxin at a dose of 10 mg / kg orally for 30 days. Experimental group 3 received diazinon toxin at a dose of 20 mg/kg for 30 days. The amount of LD50 diazinon for laboratory animals was measured orally 50-110 mg/kg orally (13). The Institutional Animal Care and Use Committee approved all animal-related protocols (Kharazmi University, Tehran, Iran).

Evaluation of changes in testicular weight and hormone level

Excess and excess lipids around the testicles, the vas deferens, and the epididymis were all removed from the environment in order to study the potential effects of diazinon toxin on testicular weight. Blood sampling was performed five days after the last administration. In order to perform the bleeding operation, the animal was anesthetized with chloroform and then by placing the animal on its back with the fingertips, the location of the heart was determined and blood was taken directly from the animal's heart. Immuno radiometric assay technique was used to measure LH, FSH and testosterone.

Assessment of sperm parameters

By using computer assisted sperm analysis, epididymal sperm parameters were evaluated (CASA). In this procedure total motile and immotile sperm, and concentration of sperm were assessed, along with concentration and sperm motility. In a nutshell, the complete epididymis was taken out and longitudinally sliced to extract sperm from the cauda. After that, the cauda epididymis was transferred to microtubes containing 1 mL of T6 solution and 25 µL BSA (bovine albumin serum), and the microtubes were then put inside an incubator (5 percent CO2 at 37 °C) for 30 minutes, or until spermatozoa formed a sperm suspension.

Assessment of sperm survival

50 µl of the sperm suspension and 50 µl of the 0.5 percent eosin dye were combined in a microtube. After shaking for 30 seconds, 100 µl of nigrosin was added to the initial mixture. A coverslip was put on a slide after a drop of the stained sperm suspension was deposited there. The x400 objective of a light microscope is used for observation. Live spermatozoa had a blue border, whereas dead spermatozoa appeared purple.

Statistical analysis

One-way ANOVA and Tukey post hoc tests were used to examine the data in SPSS version 19. P value of 0.05 above was deemed significant. The mean and standard deviation of all data were displayed (SD).

RESULTS

Diazinon's effects on sexual hormones and testicular weight

The results obtained from testicular weight show that the mean weight of testes in the experimental groups decreased compared to the control group and this reduction was significant only in group 3 (treatment with experimental dose / 20 mg / kg diazinon toxin) Table 1. With increasing doses of diazinon toxin, the mean serum testosterone decreased, and the difference between the means of experimental groups 1 (treatment with an experimental dose of 5 mg/kg diazinon toxin), 2 (treatment with an experimental dose of 10 mg/kg), and 3 (treatment with an experimental dose of 20 mg/kg diazinon toxin) is significantly smaller than that of the control group (Table 1). FSH significantly increased in comparison to control group between experimental groups 2 (treatment with experimental dosage of 10 mg/kg diazinon toxin) and 3 (treatment with experimental dose of 20 mg/kg diazinon toxin). There was no significant alteration between experimental group 1 (treatment with experimental dose of 5 mg / kg diazinon toxin) and placebo group in compared to control. In the study of mean serum LH between experimental groups 3 (treatment with experimental dose of 20 mg / kg diazinon toxin) with the control group, a significant increase was observed. The results are presented in Table 1.

Table 1: Comparison of the mean effect of diazinon toxin on testosterone, FSH, LH, and testicular weight in adult male mice

Group name	Testosterone (ng/ml)	FSH (mIU/ml)	LH (mIU/ml)	Testicular weight
Control	2. 55 ± 0.73	0.9075 ± 0.27	1.1037 ± 0.52	1.430 ± 0.099
Placebo	2.4 ± 0.65	0.9121 ± 0.26	1.109 ± 0.54	1.401 ± 0.091
Group 1 (5 mg/kg)	1.2863 ± 0.49*	0.9563 ± 0.302	1.1612 ± 0.52	1.3473 ± 0.093
Group 2 (10 mg/kg)	1.37 ± 0.43 *	1.5025 ± 0.56 *	1.3850 ± 0.52	1.2976 ± 0.16
Group 3 (20 mg/kg)	0.7613 ± 0.08 **	1.9938 ± 0.33 *	1.8038 ± 0.47 *	1.1743.99

FSH: follicle stimulating hormone, LH: luteinizing hormone. Data represent as mean ± SD * p<0.05.

Evaluation of sperm parameters

The computer-assisted sperm analysis revealed that, in comparison to the control group, the sperm concentration and mobility (progressive, nonprogressive, motile, and immotile) were significantly lower in experimental groups 1 (treatment with experimental dose of 5 mg / kg diazinon toxin), experimental 2 (treatment with experimental dose of 10 mg / kg diazinon toxin), and experimental 3 (treatment with experimental dose of 20 mg/kg diazinon toxin). The fraction of motile sperms significantly decreased in the diazinon groups compared to the control group (p <0.05). Between the control group and placebo group, there was no discernible difference. Additional modifications to sperm properties are listed in Table 2.

Sperm viability after diazinon therapy

Figure 1 depicts the findings of the eosin-negrosin staining, which reveal a substantial difference between the control group and the diazinon groups (5, 10, 20 mg/kg) in terms of sperm viability. After 30 days of oral diazinon therapy (5, 10 and, 20 mg/kg), compared to the control group, the proportion of epididymal sperms that were alive was significantly reduced (50% ± 3.4, 45% ± 5.5 and, 30%± 2.21 respectively) (p<0.05). There was no significant difference between control and placebo groups.

DISCUSSION

An organophosphate chemical called diazinon is often used in domestic and agricultural settings. It has detrimental impacts on the health of living things (14). Pesticide residues in the environment and food items are a concerning indicator that require scientific evaluation at the preclinical and clinical levels (15). Recently, there has been a sharp decline in semen quality. By altering the DNA or proteins bound, DZN can harm testis cells and cause mutations in spermatogonia, which ultimately result in alterations in the sperm. The present study investigated the induced testicular toxicity by DZN on the male adult rat (16). For this purpose, rats were treated with DZN (5, 10 and 20 mg/kg) for 30 days. The results of the current investigation show a considerable decrease in the number and motility of sperm in the rats exposed to diazinon in group 3 (20mg/kg). However, the effect of diazinon on different groups depended on the received dose but a decrease in sperm count was observed in all three groups. Among the 5rat receiving the 5 mg/kg of DZN, sperm count and motility were slightly reduced in all of them. Among the rat receiving a dose of 20 mg/kg of DZN, 3 of them had a significant decrease in sperm count. All assessed motility metrics show a considerable reduction in CASA-based sperm motion analysis in group 3. Also, compared to the control group, sperm movement was reduced in the first and second groups (5, 10 mg/kg). But the highest number of immobile sperms was seen in the group 3 at the dose of 20 mg/kg. Also, our research showed that the weight of the testicles in 3 out of 5 rats receiving a dose of 20 mg of DZN was greatly reduced, while in the other 2 rats, a slight change was observed. The weight of the testicles in the groups receiving the dose of 5 and 10 mg/kg of DZN was associated with a small change.

Table 2. Sperm parameters after being treated with different dose of diazinon toxin.

Parameters	Control	Placebo	Group 1 (5 mg/kg)	Group 2 (10 mg/kg)	Group 3 (20 mg/kg)
Volume (Ml)	80.125 ± 7.73	79.791 ± 7.34	49.25 ± 19.22 *	42.5 ± 10.75 *	35.87 ± 6.08 **
PR (%)	40.34 ± 6.15	35 ± 8.23	22.2 ± 4.29 *	20 ± 5.78 *	15 ± 7.34 **
NP (%)	15.44 ± 7.38	14.58 ± 6.64	13.58 ± 3.96	11 ± 5.64 *	10 ± 5.6 **
IM (%)	30.56 ± 4.15	37.59 ± 8.76	44.62 ± 10.37 *	50 ± 7.5	59 ± 4.78 **
M (%)	57.10 ± 5.15	59.31 ± 10.76***	51 ± 9.26 *	47.62 ± 7.89	41.5 ± 7.9**

PR, progressive, NP, non-progressive, IM, immotile, M, motile. Data represent as mean ± SD * p<0.05.

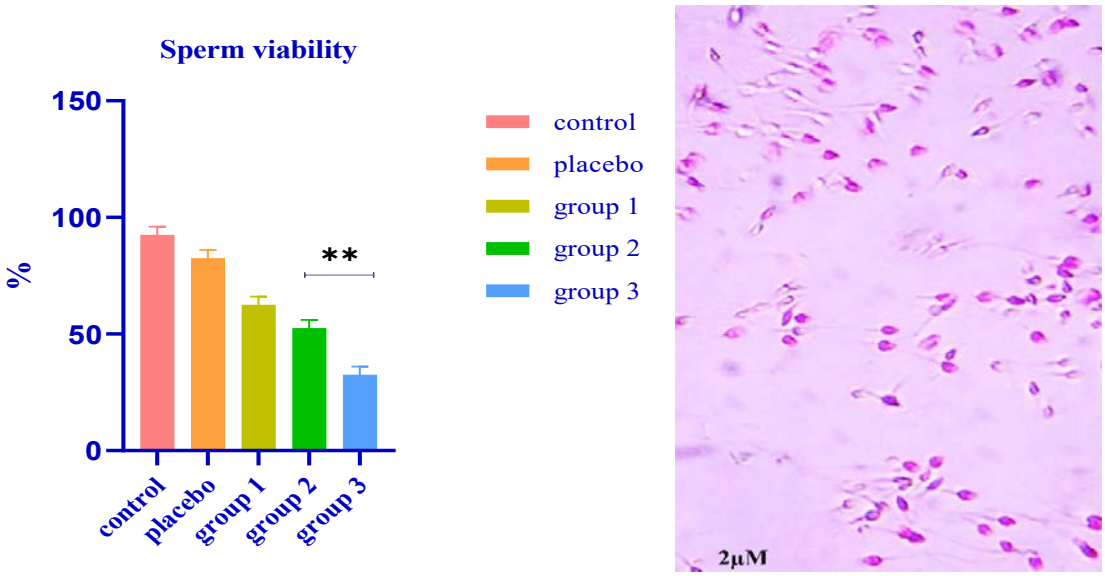

Figure 1. Assessment of sperm viability by eosin-nigrosin staining. The results showed a significant decrease of sperm viability in the group 3 (treatment with experimental dose of 20 mg/kg diazinon toxin). Live sperm look white, whereas only dead sperm are stained with eosin, making them a dark pink color.

Our results show that the effects of DZN on testis weight, sperm count and motility may be different among different rats.

Additionally, a higher percentage of sperm with pink heads and a higher percentage of dead sperm dramatically increased in diazinon administration groups. Our results showed that the highest amount of dead sperms was in the group receiving a dose of 20 mg/kg of DZN. Also, our microscopic evaluation showed that among 5 mice receiving 20 mg/kg dose of DZN, 3 of them had higher percentage of dead sperms. These results concur with those from human and animal research studies that have been published. In this investigation, rats given oral doses of 5, 10, and 20 mg/kg diazinon for 30 days showed decreased sperm concentration and motility, decreased testicular weight, and an increase in dead sperm. Numerous processes might account for the decreased sperm quality brought on by DZN (17). Reduced sperm quantity, movement, and shape, as well as other reproductive abnormalities, may be caused by the modification in sperm parameters, which has a direct impact on testicular tissue (18). According to certain research, DZN can cause biochemical abnormalities in the ovaries and testes as well as morphological and functional alterations (19). Based on particular research, a low testicular Leydig cell count is linked to low testosterone synthesis, which might lead to spermatogenic deficits (20). Our findings show that sublethal doses of diazinon administration can negatively affect reproductive function by reducing testicular bulk and blood levels of testosterone while raising circulating gonadotropin concentrations.

The loss of all types of seminiferous tubules and spermatogenesis may be caused by diazinon therapy, according to several histological investigations conducted on animals (21). Since maintaining LH serum levels is crucial for starting and supporting spermatogenesis, excessive levels of circulating LH may contribute to Sertoli cell and germinal cell degeneration (22). Among the different groups, serum LH and FSH levels were associated with a significant increase in the group treated with a dose of 20 mg/kg of DZN. Serum LH and FSH levels were increased in 4 out of 5 rats in this group as expected. Also, serum testosterone among different mice of this group was reduced with a small difference. Studies demonstrate that OP disrupts the epithelium's microtubules, which ultimately results in tubular atrophy. Additionally, interruption of spermatogenesis may result from elevated serum LH levels, which are harmful to germinal cells (19). Also, the fact that severe gonadotoxicity might be observed with extended exposure to DZN was demonstrated by a decrease in testis weight. OPs have a history of disrupting reproduction. In rats exposed to OP pesticides, broken sperms, cytoplasmic vesicles, and impaired sperm movement are the main indicators of lower quality (23). Reduced sperm counts were discovered in Chinese pesticide manufacturing employees by Pedungtod et al. in 2007. According to observations, OPs can cause oxidative stress by changing the activity of the free radical scavenging enzymes (24). The toxicity of different pesticides may be influenced by the reactive oxygen species (ROS) that OP causes. The damaging effects of ROS on reproductive tissue may increase as a result of decreased antioxidant effectiveness. DZN administration increased lipid peroxidation (LPO)

in rat erythrocytes, as demonstrated by Sutcu et al. A causal connection is thought to exist between the high levels of polyunsaturated fatty acids (PUFA) found in spermatozoa's plasma membranes and the low levels of scavenging antioxidants found in their cytoplasm. As a result, it is speculated that one of these pathways that warrants further research may be the oxidative damage caused by DZN.

CONCLUSION

Diazinon has significantly impacted the reproductive system in male rat, in conclusion. The current findings show that DZN exposure directly affects rat testicles, and an imbalance in the levels of circulating testosterone and gonadotropins may lower fertility. Couples trying to get pregnant should get individualized advice addressing any potentially detrimental occupational exposures, notwithstanding the fact that further study is needed to explain and enhance this finding in relation to human reproduction. Medical research still has opportunity for advancement in the area of male infertility. In order to obtain the best diagnostic and therapeutic advancements for each individual, personalized medicine and its possible focused treatment of male infertility were introduced in this study.

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http://pmjournal.ir Review Article

Spring 2022, Volume 7, Issue 25 (36-42)

Alternatives to Antibiotics GOAL: ELEVATING Antibiotic Resistance During the Post-COVID Period

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DOI: 10.22034/pmj.2022.253553

Submitted: 2022-02-05
Accepted: 2021-05-24

Keywords:
Antibacterial resistance
COVID-19
Phyto-therapy
Phage-therapy

Abstract:

The COVID-19 outbreak offers an unmatched chance to take advantage of personalized medicine's benefits for the protection, detection, medication, monitoring, and administration of a fresh public health crisis. Antibiotics, which were formerly regarded as miracle cures and among the most difficult life-saving discoveries of the twentieth century, are now posing a hazard to society as a result of overuse and abuse. Antimicrobial resistance (AMR) is a widespread issue that is becoming worse, and the current COVID-19 pandemic might make things even worse. It has been shown that a significant portion of Covid-19 patients gets secondary microbiological infections. The medical industry is now facing difficulties because of this. As a result, several non-antibiotic techniques have been sought, and their processes have been examined, to slow the spread of AMR.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) was classified as a pandemic by the World Health Organization in March 2020 (1). The majority of Coronavirus (COVID-19) individuals globally are most susceptible to secondary infections. Antimicrobial resistance (AMR) has increased as a result of the introduction of COVID-19 due to an increase in microbial pathogens as secondary infections (2,3). The World Health Organization has stated that by 2050, the number of human fatalities brought on by drug-resistant bacteria might increase from around 700,000 to 10 million (4). The biggest contributor to AMR has been the increasing use of antibiotics during covid outbreaks. However, high rates of incorrect antimicrobial prescription, improper use of biocides, and discontinuation of therapy for other disorders may be to blame for the establishment of antimicrobial resistance. Antibiotic use has increased, which has contributed to the creation and spread of antimicrobial resistance (AMR), a significant worldwide health issue. Antimicrobial stewardship programs include minimizing the use of antibiotics as one of their key suggestions for combating AMR (5). A concerning biological issue is the inability of some illnesses to be treated by currently available antibiotics (6,7). The need of integrating antimicrobial stewardship practices with the healthcare system's COVID-19 response has been emphasized by the World Health Organization. Researchers and doctors are scrambling to identify a medicine that may exert antiviral efficacy with minimal side effects and should be inexpensive in the wake of the COVID-19 epidemic (8). Since SARS-CoV-2 is a recently discovered virus, repurposed medications are the only effective treatments available outside vaccinations. Based on our prior experiences with these antiviral medications against extremely pathogenic RNA viruses including HIV, Ebola, influenza, etc., clinical studies are now being done.

In addition to antiviral medications, several clinical investigations have found that COVID-19 causes an increase in cytokine and chemokine production (9), and as a result, immuno-modulatory US FDA-approved medications may also be able to help reduce the abnormal inflammatory immune response. Overall, it is recommended that antiviral and anti-inflammatory medicine combination treatment may be able to mitigate the current COVID-19 pandemic (10).

Antibiotic Resistant Emergence Favouring Factors

•enhancing environmental use of bactericidal agents
•A halt to research into other infectious illnesses
•A halt to research into other pathogens
•Shortages of medications, particularly narrow spectrum antimicrobials, and personal protective equipment (e.g., hydroxychloroquine);
•Scarcity of medications, particularly limited spectrum antimicrobials, and personal protective (e.g., hydroxychloroquine);
•Overpopulation and overburden of medical systems (11).

Most antibiotic medications are used as prophylaxis to shield hospitalized patients from further bacterial infections. The widespread issue of antibiotic resistance is not the only one. Changes in the gut flora may be another unfavourable impact of antibiotic therapy in patients. Furthermore, individuals with COVID-19 may experience poorer outcomes as a result of gut microbiome abnormalities. It's interesting to note that COVID-19 patients with GI issues have been reported to have more respiratory discomfort than COVID-19 patients without GI symptoms (12).

Anti-Microbial Resistance (AMR)

using cleaning products and washing hands

While suffering from a covid-19 infection, using hand sanitizers and disinfectants commonly exposes the user to both pharmacological and non-pharmaceutical substances in various amounts (13). Phenol and hydrogen peroxides, which cause bacterial DNA damage, are included in the majority of commercial sanitizers (11–13).

Antimicrobial resistance gene transmission mechanisms

Inhibiting or restricting drug absorption, bypassing the pathway (compensatory tack) impeded by a drug, demeaning and inactivating a drug by modification/degradation enzymes, and pumping a drug out of the body by different types of active efflux pumps are the primary mechanism of resistance to antibiotics. Modifications in cellular membranes that reduce the permeability of the membrane and alteration in cell wall proteins are the prevalent antibiotic targets (14).

Therapies without antibiotics to target AMR

To solve the AMR issue, suitable antimicrobial alternatives must be taken into consideration. Probiotics, phages, and phytomedicines are a few non-antibiotic methods for treating and preventing different illnesses (15).

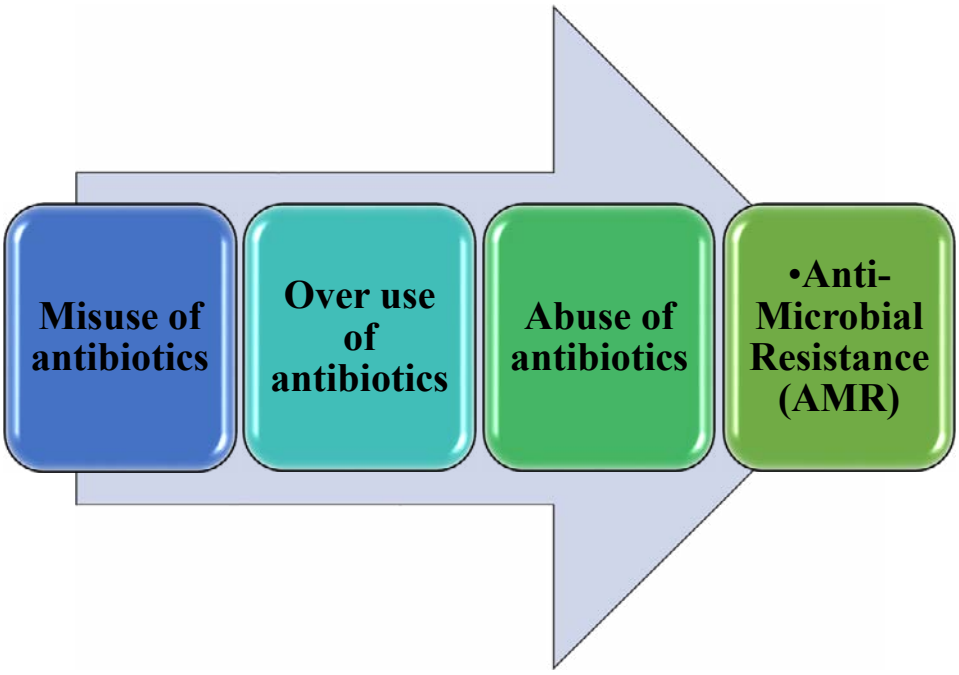

Fig1. Development of Antibiotic Resistance.

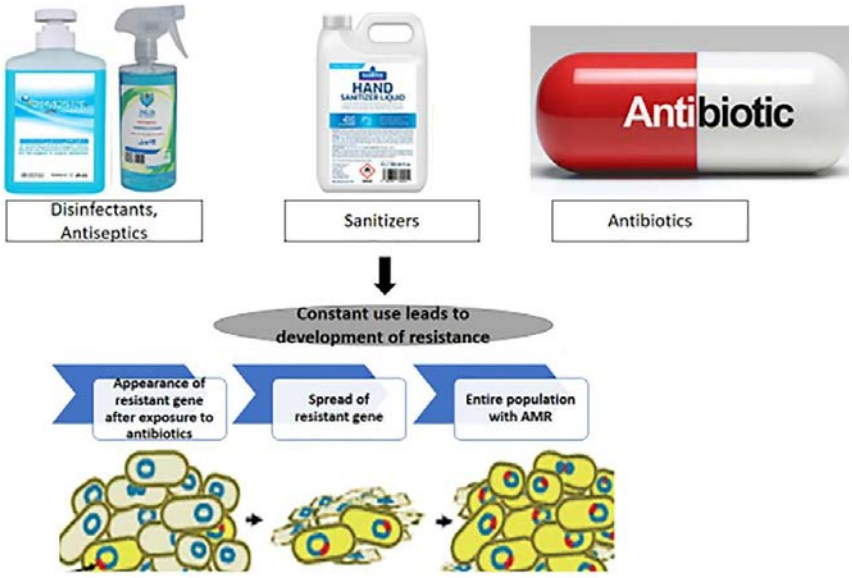

Fig2. Antimicrobial resistance gene transmission mechanisms.

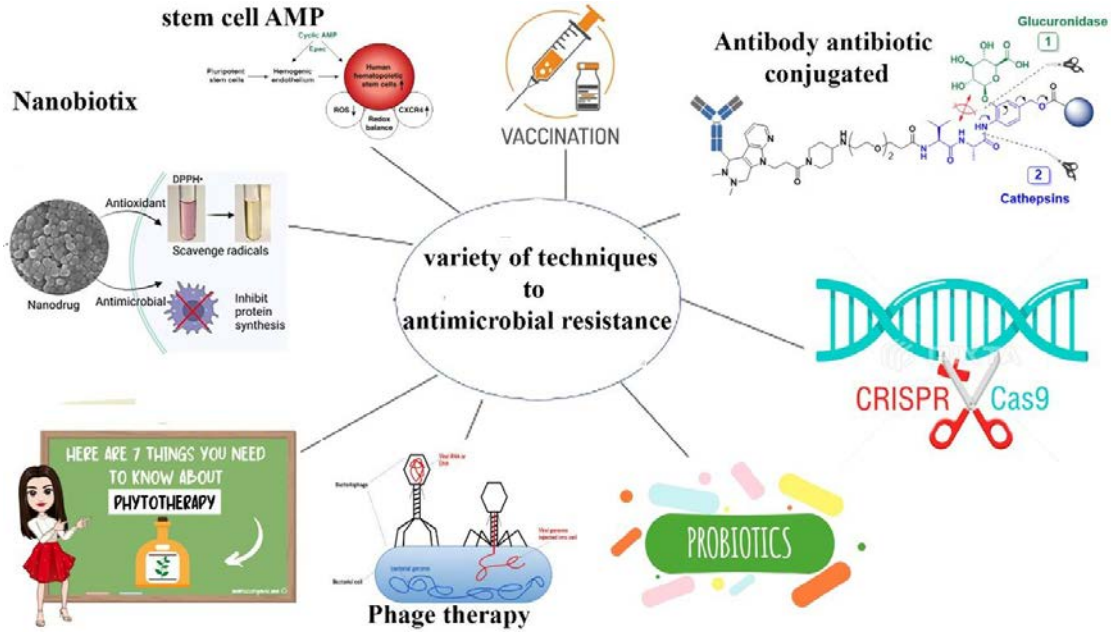

Fig3. Multiple strategies are used to address antimicrobial resistance.

The probiotic and prebiotic

Probiotics have been used as an alternative therapy for several intestinal illnesses, including gastroenteritis and diarrhea brought on by antibiotics. By granting immunity to infection or getting rid of infectious agents, probiotics have a positive impact on the digestive and other systems. As probiotics, several bacterial and yeast species have been employed (16).

Prebiotics are non-absorbable polysaccharides (like inulin and fructo-oligosaccharides) that promote the variety of the human gut microbiota and demonstrate health advantages in the host. According to studies, giving prebiotics to individuals who suffer diarrhea brought on by antibiotics has worked well (17).

Antimicrobial agents based on Bacteriophages

Bacteriophage treatment involves lysing bacterial pathogens with phages. Bacteriophage treatment has gained increased attention as antibiotic resistance has become a significant issue in contemporary medicine (18).

Phytomedicines

Bioactive substances that are obtained from plants

are known as phytomedicines. To treat diverse illnesses, several phytocompounds are utilized as lead molecules. Approximately 25% of all prescription pharmaceuticals used in the USA contain one or more bioactive chemicals derived from vascular plants. It is estimated that more than two-thirds of the world's population now depends on plant-derived medicines (19). According to estimates, plant ingredients make up or have served as models for 50% of current Western pharmaceuticals (20). Many of the commercially successful medications used in contemporary medicine were first used in undeveloped forms in conventional or folk medicine, or for other uses that showed potential biological activity. Additionally, researcher (21) found that extracts from 15 widely used Indian medicinal herbs were effective against enteric bacteria that produce ESBLs and are multidrug resistant. 45 Iranian medicinal herbs were shown to have an action against many drug-resistant human diseases, according to researches (22). Additionally, beta-lactamase generating methicillin-resistant Staphylococcus aureus was observed to be affected by several bioactive plant extracts (23). Several studies have been conducted to support the claims made for alternative treatments.

Faecal microbiota transplantation (FMT)

FMT involves transferring a healthy person's feces to a patient to repair the patient's damaged gut flora. According to research, this FMT can cure infections brought on by vancomycin-resistant enterococci or multidrug-resistant K. pneumonia, two examples of drug-resistant microorganisms (24).

Stem Cell-Derived Antimicrobial Peptides

Mesenchymal stem cells (MSCs) have undergone substantial research to provide a safe and effective therapeutic solution for several chronic disorders. MSCs have remarkable potential for enhancing immunomodulation, tissue repair, and inflammation management (25). According to a recent investigation, human MSCs behave as antimicrobial peptides (AMPs) that kill bacteria in a variety of ways, including preventing the formation of bacterial cell walls (26).

Blood Filtration Variations

In some illnesses, controlling the cytokine storm is crucial to avoiding organ damage. Devices are used in hemofiltration or renal replacement therapies to bind to and remove circulating bacterial products, inflammatory mediators, and cytokines (27) as well as some pathogens. Mannose-binding lectins and bound heparin are two of the frequently used tools (28). It is believed that even in cases of multidrug resistance, the host immune system will be able to combat the remaining pathogens if a significant reduction in the pathogenic bacterial load is achieved by this hemo-filter (29).

Quorum sensing blockers

The two crucial characteristics of microbial pathogens boosting their survival chances in harsh conditions are the ability to form biofilms and quorum sensing. Numerous organic and synthetic compounds have been demonstrated to inhibit quorum sensing (30).

Role of CRISPR-Cas against AMR

In microbial species, CRISPR-cas is a particular adaptive immunological characteristic that offers defence against invasive bacteriophages (18, 31).

Significance of Nano-antibiotics to combat AMR

It is possible to use nanoparticles to deliver antimicrobial agents or for them to already contain such agents. Due to their improved antimicrobial and anticancer activity and low toxicity, metal and metal oxide-based nanomaterials and drugs are viewed as attractive therapeutic options for use in biological sciences in the future (32). Through a variety of mechanisms, including bacterial wall disruption, biofilm suppression, immune response activation in the host, production of reactive oxygen species, and damage to important DNA and protein molecules of the resistant bacteria, nanoparticles can be used as carriers for the delivery of drug candidates and also have antimicrobial effects (33).

Change in the microbial community

The entire number of microbes present in a person's body is called their microbiota, and their microbiome contains all of their genomes. More than 160 bacterial species, primarily Bacteroidetes and Genera, have been linked to the regulation of physiological processes in a healthy adult gut. Many disorders, including diabetes, cardiovascular disease, asthma, autism, inflammatory bowel disease (IBD), antibiotic-associated diarrhea, and cancer, have been linked to the dysbiosis of this ecosystem. diseases, asthma, autism, inflammatory bowel disease (IBD), antibiotics-associated diarrhoea and cancer (34).

Personalized medicine and the treatment of the corona virus

The coronavirus cannot currently be treated with a particular antiviral medication. The only medications that have only a marginal impact on the coronavirus are recombinant IFN interferons and ribavirin. This is especially true for the brand-new coronavirus COVID-19. Because of this virus's mutation, which affects a crucial enzyme as a receptor, therapy is more challenging (35). Numerous anti-coronavirus medications have been created in response to the coronavirus epidemics caused by SARS and MERS,

but none of them have yet completed clinical trials. These medications target coronavirus enzymes such as proteases, polymerases, and MTases, as well as entrance proteins. They have encountered failure (36). The major therapy up till now has been suggested to use antibodies and plasma from recovered patients. Given the intense epidemic character of COVID-19 and its low fatality rate, this plasma and antibody collection from recovered patients may be the sole treatment option available right now for people with the disease. As coronaviruses are not curable or preventable, the best strategy to handle a serious coronavirus infection is to restrict the source of infection, receive an early diagnosis, supportive therapies, and prompt information distribution, rather than spreading fear. The outbreak must be controlled with panic (37). This virus can be prevented from spreading via preventative measures including good personal cleanliness, using an appropriate N95 mask, ventilating enclosed spaces properly, and avoiding needless transportation in congested areas (38). Currently, outpatient diagnostic and treatment services and inpatient services can be used to classify therapeutic services. When personalized medicine was implemented in Covid19, activities were created to specify how genomes, evolutionary biology, metabolomics, and viral genomes interacted to create events like infection, serious infections, therapeutic response, and sensitivity to immunization (39). In order to manage specimens in the strategy to personalized medicine in COVID-19, a joint directive from the Secretary General for Research, Development, and Innovations in Health and the Executive Directorate of the Andalusian Health Service was implemented on January 2020. Additionally, medical practitioners will have electronic biochemistry request accessibility to the whole genomic research of the SARS-CoV-2 virus (MPA) (40).

CONCLUSIONS

The development of alternative medicines is advised to lessen reliance on chemical medications because antibiotic resistance might prove fatal. Antibiotic effectiveness is decreasing as a result of the rise of drug resistance. Therefore, it is crucial to find new approaches and therapies to address the issue and cut down on the usage of antibiotics. The epidemic has forced us into a new situation that encourages collaboration and relationships between states and research institutes. Professionals have also arisen to treat this complicated illness, and technology has been introduced to ensure home health care. Understanding the interplay between the virus and the host might be improved with the use of sequence analysis, bioinformatics, and medical professionals focusing on tailored treatment. Physicians ought to have access to these technologies and be able to use them in their regular decision-making processes. Big data, AI systems, and the growing need for customized treatment are enabling the development of algorithms based on individual factors (genomic), the host, and the guest (pathogen and patient subject).

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Spring 2022, Volume 7, Issue 25(43-47)

TGF-β Isoforms and Receptors: A Gene Expression Analysis in Multiple Sclerosis Patients and Normal Individuals

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DOI: 10.22034/pmj.2022.253554

Submitted: 2022-01-03
Accepted: 2022-04-28

Keywords:
TGF-β Pathway
TGF- β receptors
Multiple Sclerosis
Personalized Medicine

Abstract:

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), depicted by lymphocytic infiltration and demyelination. MS is associated with the up-regulation of pro-inflammatory and down-regulation of anti-inflammatory cytokines. The purpose of this experimental study was to evaluate the expression level of TGF-β1, TGF-β 2, TGF-β-R1, and TGF-β-R2 mRNAs in peripheral blood mononuclear cells (PBMCs) from MS patients and healthy controls using Real-Time PCR. Our findings indicated that the TGF-β-R1 expression level was 2.25 times higher in controls than in MS patients. Also, a significant correlation between normalized expression of TGF-β-R1 and TGF-β1, or TGF-β2 was observed. Therefore, these genes could likely play an important role in the etiology of MS.

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory disease, characterized by lymphocytic infiltration and demyelination in the central nervous system (CNS) (1-3). Prognosis, diagnosis, and treatment of this disorder rely upon a greater determination of the mechanisms underlying MS onset and development (4). Inflammatory cascade and the role of different immune cells (including macrophages, natural killer cells, and certain lymphocyte populations) are two considerable factors in the etiology of this disease (5).

In MS dependent inflammatory cascade, various cytokines are up-regulated or down-regulated, including pro-inflammatory (e.g. IFN-γ, TNF-α, and IL-12) and anti-inflammatory (e.g. IL-10) cytokines (6). TGF-β, as one of the most critical anti-inflammatory factors, is secreted not only by the immune cells (mainly macrophages) but also the other non-hematopoietic cells (7). The role of transforming growth factor- β (TGF-β) has been studied in several disorders, particularly in many autoimmune diseases like MS (8-10).

This cytokine is a protein present in three isoforms: TGF-β1, TGF-β2, and TGF-β3 (11). All of the TGF-β isoforms could participate in the generation of chronic MS lesions (12). Mechanistically, TGF-β signals are generally initiated through binding this factor to the heterodimer complex, composed of type I and II trans-membrane receptor serine/threonine kinase, transforming growth factor-β receptor 1 (TGF-β-R1) and TGF-β-R2 (13). Thus far, several functions have been determined for TGF-β in MS pathogenesis, including 1) suppressing the immune responses, 2) inhibiting T cells, B cells as well as many other cells, 3) inducing the production of regulatory T cells (Treg), 4) repressing leukocyte adhesion to endothelium, 5) down-regulation of adhesion molecules, and 6) sustaining a state of immune tolerance (14, 15).

With regards to the importance of TGF-β's role in MS pathogenesis, in this experiment, we evaluated the gene expression level of two relevant isoforms (TGF-β1 and TGF-β2) as well as receptors (TGF-β-R1 and TGF-β-R2) in peripheral blood mononuclear cells (PBMCs) of MS patients, in comparison with normal individuals. In addition, we investigated the correlation of the gene expressions with the expanded disability status scale (EDSS), the age of onset, or the disease length.

METHODS AND MATERIALS

Patient and control participants

This experimental study was conducted in the genetics Laboratory of Tarbiat Modares University (Tehran, Iran) from 2013-2014. In this research, 61 MS patients and 36 age-, race-, and sex-matched controls were recruited from Multiple Sclerosis centers at Sina Hospital (Tehran, Iran) to investigate the mRNA expression level of TGF-β1, TGF-β2, TGF-β-R1, and TGF-β-R2 genes. The patients were diagnosed according to the McDonald criteria. informed consent was obtained from all human adult participants and the study was approved by the Ethics Committee of Tarbiat Modares University (ethical code: d52/6723).

RNA extraction and cDNA synthesis

After blood collection, PBMC separation was performed using density gradient Ficoll/Paque solution (lymphocyte, Cedarlane, Netherlands) and total RNA was extracted from all patient and control samples, using RNXTM-plus reagent (Cinnagen, Iran) according to the manufacturer's protocol. The samples were subsequently reverse-transcribed into cDNA, using 3 µg total RNA and 250 µg oligo dT (MWG, Germany). The reaction was incubated at 70° C for 10 minutes and cooled on ice for 3-5 minutes, followed by adding an RNase inhibitor, 10 mM dNTPs, and Reverse Transcriptase (all from Fermentas, Canada) to 20 ml total volume of the reaction mixture. The mixture was ultimately incubated at 42° C and 80° C for 60-90 and 15 minutes, respectively.

Quantitative reverse transcriptase PCR

cDNA for each sample was used to evaluate the mRNA expression level of TGF-β1, TGF-β2, TGF-β-R1, and TGF-β-R2 genes using relative quantitative reverse transcriptase PCR (qRT-PCR). In this experiment, glyceraldehyde3-phosphate dehydrogenase (GAPDH) was utilized as a housekeeping gene.

Each qRT-PCR reaction was performed in a final volume of 20 µl, using 10 ng cDNA, 2x SYBR Green I master mix (Takara, Shiga, Japan), and an appropriate primer pair set (Table S1). Thermal condition, as one-step RT-PCR, was carried out by an initial step at 95° C for 15 minutes, followed by 40 cycles at 95° C, 60° C and 72° C for 15 seconds, 30 seconds, and 30 seconds, respectively, in Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, USA). Termed cycle threshold (Ct) was determined for each sample, and the average Ct of duplicate samples was calculated.

STATISTICAL ANALYSIS

Relative quantification data analysis was performed using an arbitrary method (ΔCt). The significance of differences between control and test groups was determined by an independent t-test using SPSS software (Version 20; SPSS Inc, Chicago, USA) and GraphPad Prism 5 (GraphPad Software, Inc., San Diego, USA). The correlation analysis was assessed by Pearson's correlation coefficient. A P-value of 0.05 was set as a significant threshold.

RESULTS

In this experiment, the age of MS Patients was between 18 and 52 years, with a female to male ratio of 51:10. The control individuals were between 22–45 years of age, with a female to male ratio of 25:11. The demographic characteristics of patients are given in Table 1.

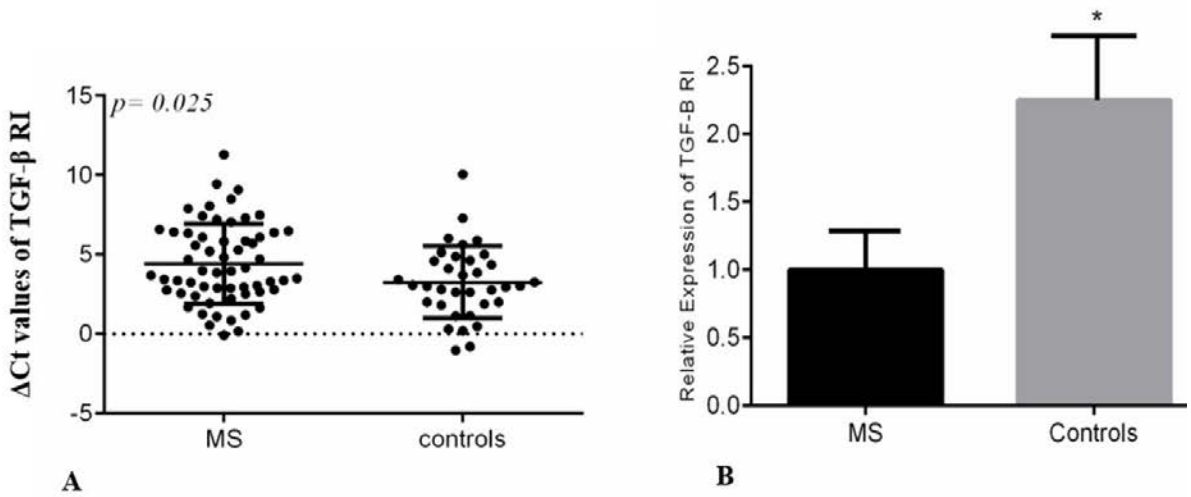

Fig.1 TGF-β-R1 gene expression in PBMCs. A) The mRNA expression of this gene shows a significant increase in the controls compared to MS patients (2.25 times). B) ΔCt values in the patient and control samples. The results were normalized relative to the level of GAPDH mRNA expression.

Table1. Demographic and disease characteristics of the MS patients and controls.

	MS Patient	Control
Number	61	31
Age (mean years ± SD)	32.54±8.45	29.03±6.81
RR/SP/PP	55/6/0	-
EDSS (mean ± SD)	2.45±1.33	-
Disease Duration	6.42±3.72	-

Table 2 Correlation between normalized TGF-β1, TGF-β2, TGF-β-R1, or TGF-β-R2 expression level in MS patients.

correlation	r	p-value
TGF-β1-TGF-β2	0.077	0.551
TGF-β1-TGF-β RI	**0.292**	**0.023***
TGF-β1-TGF-β RII	0.164	0.216
TGF-β2-TGF-β RI	**0.477**	**0.0001*****
TGF-β2-TGF-β RII	0.010	0.9939
TGF-β RI-TGF-β RII	0.194	0.143

*significant p-value < 0.05 ***significant p-value < 0.001

Gene expression analyses in MS patients compared to the controls

Findings showed that the TGF-β-R1 mRNA level mean was significantly increased in the PBMCs obtained from the controls compared to MS patients (2.25 times, p= 0.025). However, no significant difference was observed for TGF-β1, TGF-β2, and TGF-β-R2 mRNA expression levels (Fig. 1).

Analysis of the relationship between the expression levels of genes

We investigated the correlation between mean mRNA levels of the analyzed genes. There was a significant positive association between TGF-β-R1 mRNA level mean and TGF-β1, or TGF-β2 in MS patients, but no significant correlation was observed between the other genes' mRNA expression level mean (Table 2).

Moreover, we observed no significant correlation between the normalized expression level of TGF-β1, TGF-β2, or TGF-β-R1 genes and disease length, EDSS scores, or age in MS patients. In contrast, data analysis showed a significant positive correlation between TGF-β-R2 mRNA expression level and disease length in MS patients (r= 0.356, p= 0.006).

DISCUSSION

TGF-β isoforms (TGF-β1, -β2, and -β3) are signaling ligands that promote expression of the extracellular matrix protein components, control growth and differentiation of the epithelial cells and regulate immune cell function. Thus, the study of the expression of TGF-β isoforms and their receptors may be useful in determining different responses to immunomodulatory as well as combinatory therapies. Moreover, the investigation of TGF- β isoforms offers a model for personalized medicine and precision treatment in patients with different types of cancer and neurodegenerative disorders. Curiously, TGF-β isoforms are important due to their conservation among vertebrates and their different roles in a variety of human diseases including tissue fibrosis, cancer, and MS (15). CNS degeneration and inflammation are important causes of demyelination in MS(16). In 1998, Vincent et al. compared the expression of TGF-β-R1 and TGF-β-R2 by immunohistochemistry in the brain tissue of MS patients and normal controls. They determined that TGF-β isoforms were expressed in microglial cells of the normal individuals' brain tissue. They also demonstrated that the TGF-β isoforms and relevant receptors are expressed in MS lesions(17).

Expression profiling of the MS patients' peripheral blood showed misregulation of SMAD4, SMAD7, and TGF-β-R2. These findings revealed that TGF-β regulation is reduced in MS patients (18). Here, we demonstrated no significant difference in TGF-β1, TGF-β2, and TGF-β-R2 mRNA expression levels in the MS patients compared to controls, while TGF-β-R1 expression level was 2.25 times higher in normal subjects compared to the MS patients.

Several investigations have so far represented a difference in TGF-β1, TGF-β2, TGF-β-R1, and TGF-β-R2 mRNA levels in vitro and in vivo. TGF-β over-

expression in MS patients, with little disability after thymectomy, disclosed that this protein could have a favorable effect on human diseases with autoimmune backgrounds (19). Press et al. have previously examined glial TGF-β expression of different isoforms in 14 MS patients. Active lesions illustrated TGF-β2 immuno-reactivity of lesion encircling ramified microglia. On contrary, all three isoforms of this protein were expressed in the astrocytes of active white matter lesions. These results proposed that TGF-β cytokines could be locally expressed in demyelinated cells (20).

Further investigations showed that induction of rTGF-β1 suppressed IFN-γ, IL-4, IL-6, TNF-α, and perforin up-regulation in MS, but it had no effects on IL-10 or TGF-β expression. The selective prohibitory effects of TGF-β1 on pro-inflammatory cytokines expression potentially turn it into an attractive treatment for MS disease (21).

MS patients with no or slight disability showed a high level of TGF-β mRNA expression, while moderate or severe disability of MS patients was correlated with a high level of IFN-σ–positive cells. Therefore, TGF-β and IFN-σ could have dissenting effects on MS pathology. Thus, administration of IFN-σ inhibitors and/or TGF-β activators might improve MS disease treatment (22).

These differences between our results and others may be due to environmental factors including Vitamin D (23) or other drugs that patients use. We formerly studied TGF-β1 expression in 32 patients with MS and 32 healthy controls. Our findings showed no significant change in TGF-β1 expression in the MS patients compared to the control group (24), suggesting that large sample size could not affect gene regulation level.

Additionally, this study evaluated the correlation between these four gene expressions in MS patients. We detected a positive link only between the expression of TGF-β1 and TGF-β-R2 as well as the expression of TGF-β2 and TGF-β-R1 in MS patients; however, there was no significant association between the expressions of other genes. It is proposed that TGF-β-R1 might influence TGF-β1 and TGF-β2 expression by phosphorylating the receptor-specific SMADs and promoting a feedback mechanism. Altogether, the expression level of TGF-β-R1 could be considered a risk factor for MS disease which might be associated with inflammatory events in such patients. Investigations have previously implicated that the heterogeneous nature of whole-blood samples may not show the optimal level of gene expressions (25, 26), revealing some limitations in this study. Hence, assessment of gene expression at protein level MS patients' sorted PBMCs could help to better understand the role of TGF-β.

List of abbreviations

MS: Multiple sclerosis
CNS: central nervous system
PBMCs: peripheral blood mononuclear cells
EDSS: expanded disability status scale
TGF-β: transforming growth factor-β
TGF-β-R1: transforming growth factor-β receptor 1

Declarations

This study was in accordance with the declaration of Helsinki. This study was approved by the Ethics Committee of Tarbiat Modares University. The informed consent was obtained from all the participants, and informed consent obtained was written.

Consent for publication

Not applicable.

Availability of data and materials

All relevant data are included in the manuscript.

Competing interests

The authors declare that they have no competing interests.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

Z.SHF., M.B.; Performed all experiments, analyzed the data and wrote the manuscript. M.A.S.; Contributed to concept and design, manufactured the samples, and final approval of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors gratefully acknowledge contribution of the patients and institutions in this study as well as Roya Amirinejad and Fahimeh Molaahmadi for their valuable laboratory supports. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors, other than Department of Research Affairs of Tarbiat Modares University.

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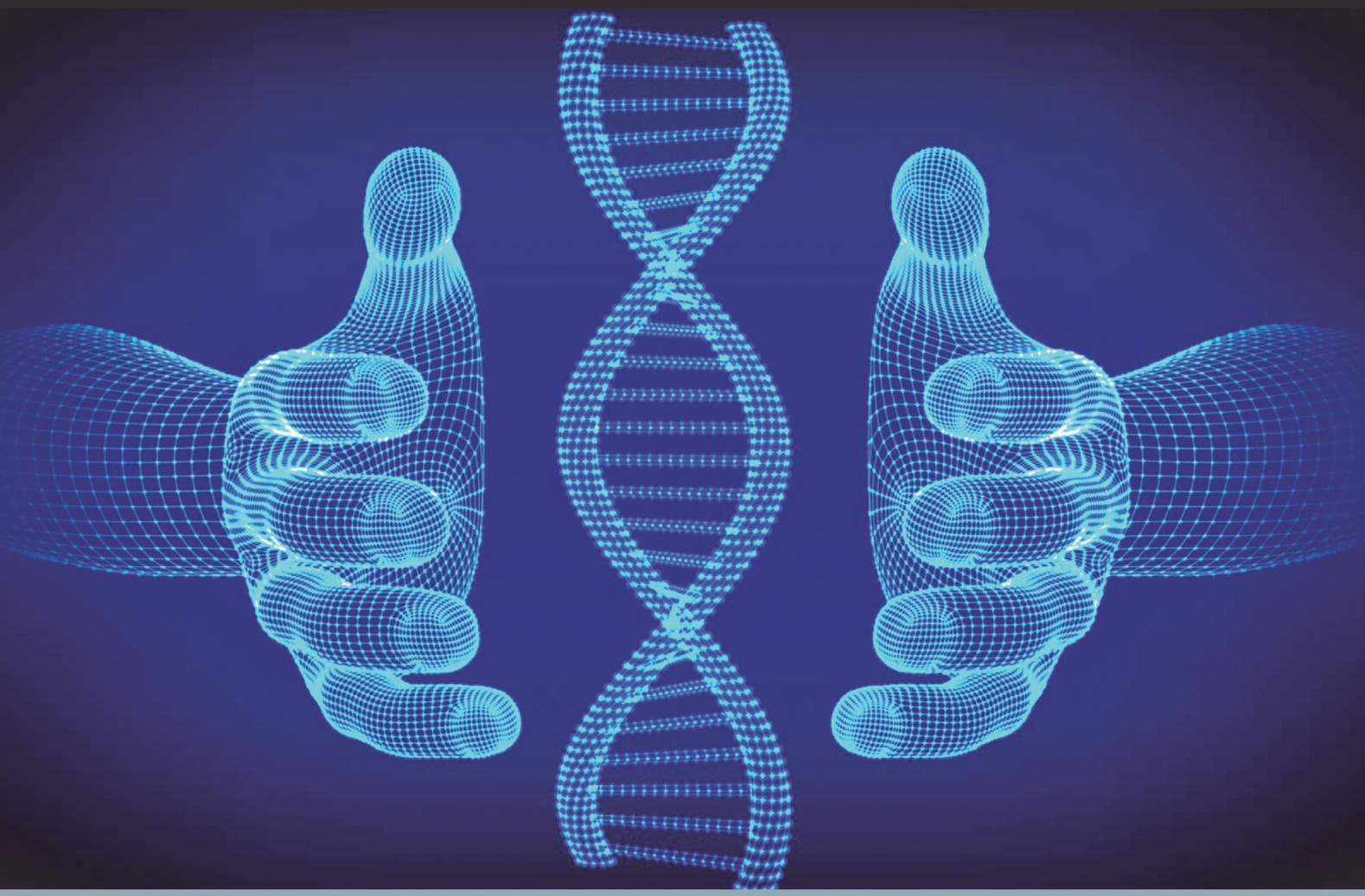

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