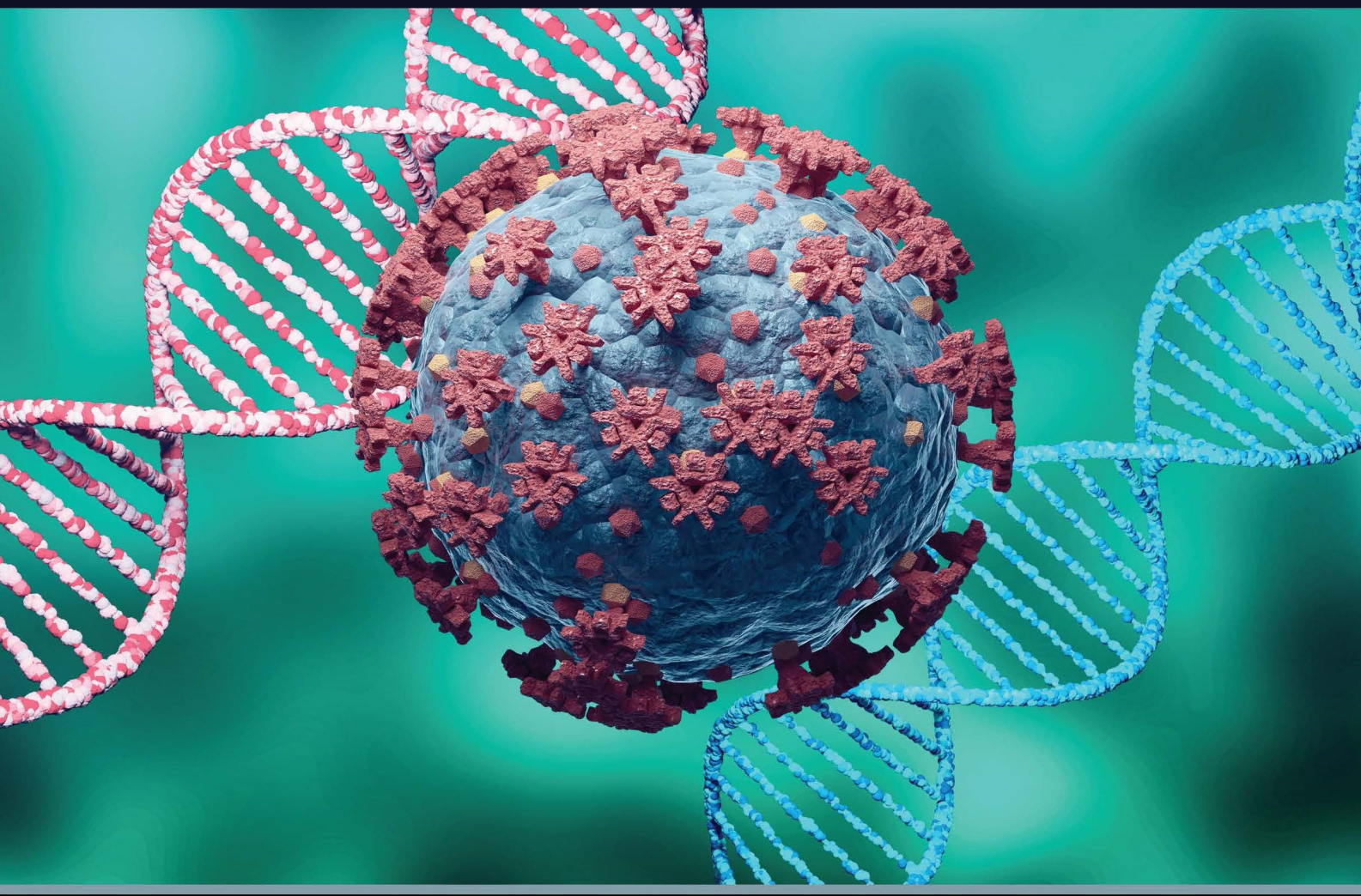
Personalized Medicine JOURNAL

Medical Journal / 8 year / No.30 / 500000 Rials / 2023 Summer / ISSN 2717-3860



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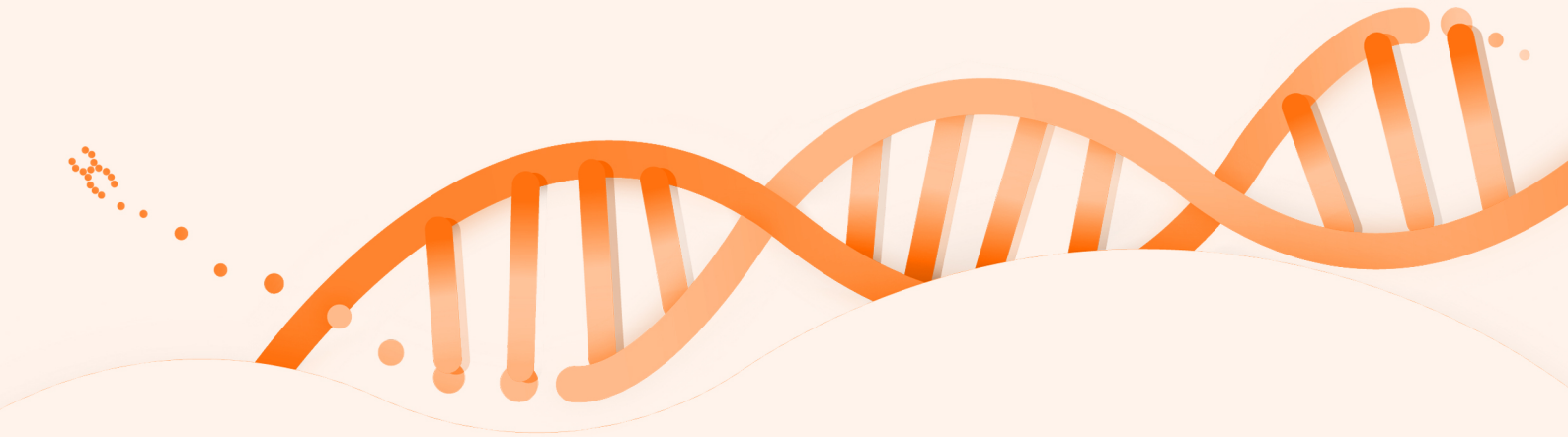

Journal Information

Name: Personalized Medicine Journal
Abbreviated Name: PMJ
Date of First Issue Published: February 2019
Concessionaire: AmitisGen TECH Dev Group
Release Period: Quarterly

Editorial Board Information

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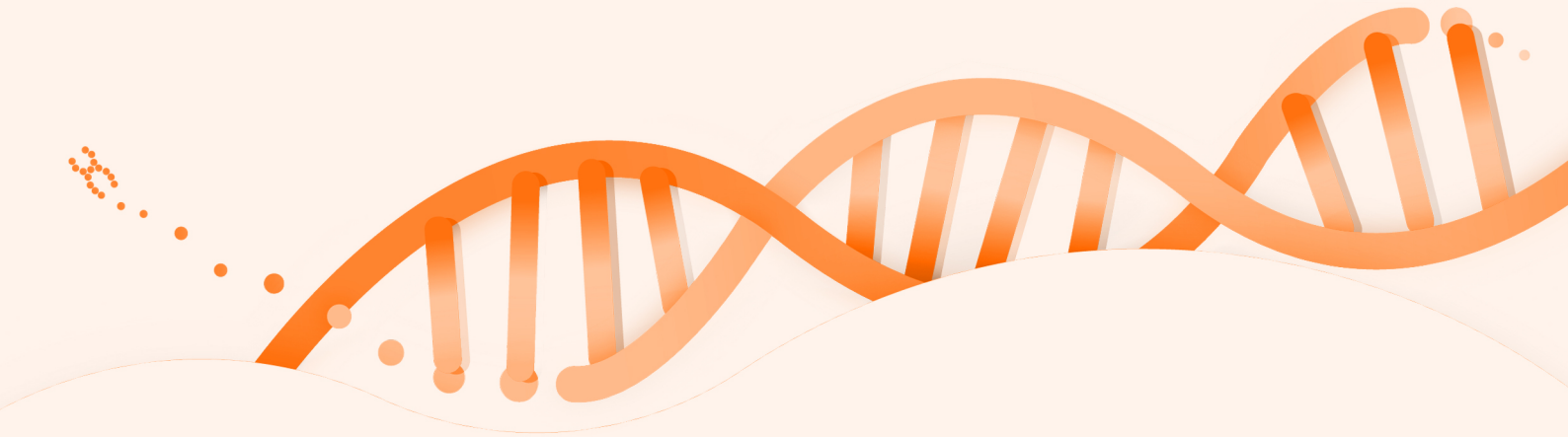

Personalized Medicine Journal
Summer 2023,Volume 8,Issue 30

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http://pmjournal.ir Original Article

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Human Amniotic Membrane Mesenchymal Stem Cells-derived Conditioned Medium Alleviates Myocardial Fibrosis

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DOI: 10.22034/pmj.2023.2007838.1007

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Submitted: 2023-07-25
Accepted: 2023-09-02

Keywords:
Human amniotic membrane mesenchymal stem cells
Conditioned medium
Heart failure
Cardiac fibrosis

Abstract:
Background: Lots of people die from heart failure (HF) because of fibrosis formation. As injured myocytes deregulated MMP-2, MMP-4, TIMP-2, Ang, plasma renin activity (PRA), and ACE leading to fibrosis, their regulation can improve HF. One of the most effective treatments for heart failure is the use of hAMSCs-CM, which has been shown to improve heart function and reduce symptoms. The study innovation was the investigation of the in vivo mode of action of hAMSCs-CM on HF fibrosis focusing on the mentioned proteins for the first time. We expected that this study partly fill the scientific gap in HF treatment.
Methods: Frothy rats were divided into 4 groups; Control, HF, culture medium, and CM. To induce HF, isoproterenol (ISO) was injected into all animals except for the control. CM were injected into the CM group and the culture medium group received culture medium. Then, cardiac functions were measured using echocardiography and serum fibrosis was evaluated by ELISA.
Results: HF model showed decreased MMP-2, MMP-4, Ang, PRA, and ACE and increased TIMP-2, whereas hAMSCs-CM therapy reversed them compared with controls.
Conclusion: Our result has partially filled the HF treatment's gap as hAMSCs-CM improved cardiac function and reduced cardiac fibrosis and the serum fibrogenic proteins.

INTRODUCTION

Annually, lots of people are dying or suffering from heart failure (HF) as the final stage of chronic heart disease all over the world (1). Ischemic heart diseases (IHD) are a leading cause of heart failure due to maladaptive cardiac remodeling (2, 3). Huge Loss of cardiomyocytes due to ischemic events activates excessive immune and inflammatory responses to protect the injured left ventricle (LV) and preserve ejection fraction (EF) (4).

As newly fibrotic tissue does not have contractile elements and interferes with normal cardiac contractile activity, it reduces ejection fraction (EF) and fraction shortening (FS) (5). It also disturbs cardiac electrical function and can cause life-threatening arrhythmia (6).

There are several treatments available to improve heart failure, but heart transplantation is the only definitive option. Pharmacological agents also failed to preserve the EF (3, 7). Therefore, an alternative strategy focusing on tissue regeneration and reducing fibrosis formation is desirable. One of the therapeutic means in regenerative medicine is stem cell therapy (8, 9).

In this regard, various cell types like mesenchymal stem cells (MSCs) and adult tissue-resident stem cells have been assessed in experimental and clinical research (8). Despite the widespread use of other cell sources, human amniotic membrane-derived MSCs (hAMSCs) are the most reliable cost-effective, and safest source of stem cells that can be used in heart diseases (10, 11). For instance, the in vitro and in vivo cardiomyogenic potency of amniotic fluid-derived

MSCs is more than bone marrow MSCs (BMMSCs) (12). In addition, the amniotic membrane is freely accessible post-parturition and hAMSCs show higher host immune system tolerance due to their unique immunological resources (13). They also possess higher proliferation and differentiation capacity, resulting from their embryonic origin such as OCT4 overexpression. MSCs produce and secrete paracrine factors known as conditioned medium (CM) which comprises a diverse range of cytokines, growth factors, and therapeutic peptides and proteins (14). A lot of evidence has shown that treatment with CM, instead of direct MSCs, makes up the MSCs-related drawbacks such as tumorigenicity and is more time and cost-effective (11).

The mechanism of LV remodeling which leads to ischemic-related HF has not been understood. However, studies showed that LV stiffens and remodeling is vastly associated with extracellular matrix (ECM) degradation (6). ECM is produced and released by injured myocytes and endothelial cells and is regulated by matrix metalloproteinases (MMPs) in which MMP-2 and MMP-9 are involved in LV changes related to acute myocardial infarction (AMI) (15). Based on evidence, screening the plasma MMP-9 can be a mortality predictor in Congenital heart disease (CHD) (16). MMP-2 can be also considered as an HF biomarker, as higher plasma MMP-2 was associated with congestive HF (17) and significantly predicted HF with Preserved Ejection Fraction(HF-PEF) with 91% sensitivity and 76% specificity (18).

Additionally, Tissue inhibitors of metalloproteinases (TIMPs) are important regulators of the MMPs as ECM-degrading enzymes (19). TIMP-2 levels are altered in patients with certain heart conditions, such as pressure overload and atrial fibrillation, but not in those with ischemic or idiopathic dilated cardiomyopathy (19), In end-stage dilated cardiomyopathy, TIMP-2 levels were found to be elevated, suggesting a potential role in disease progression (20). TIMP-2 is the most important TIMPs because it prohibits as well as activates some MMPs. For instance, TIMP-2 is necessary for cell surface activation of MMP-2 via converting pro-MMP-2 to active MMP-2 with lower molecular weight. MMP-2 also is associated with several cardiomyopathies (19). So, TIMP-2 overexpression in HF is very important because of its dual biochemical function.

In addition, plasma renin activity (PRA) levels have been associated with an increased risk of cardiovascular events (21). PRA is also introduced as a prognosticator in HF patients with LVEF. Acute decompensated heart failure (ADHF) people with elevated PRA on admission represent poor prognosis. PRA significantly fluctuates with alteration in fluid volume and renal blood flow after HF therapy (22). The baseline PRA at the admission of acute HF people was an independent prognostic marker for their readmission as well as mortality (21).

Furthermore, angiotensin-converting enzyme 2 (ACE2) plays an important role as a counterfactor in adjustment of the renin-angiotensin-aldosterone system (RAAS). Renin cleaves angiotensinogen to AngI, which is more catabolized by ACE to AngII (23).

AngII can lead to some conditions such as hypertension and fibrosis. ACE2 is also considered a key mediator in human HF, hypertension, and different cardiovascular problems (23). Higher levels of ACE2 is associated with more vulnerability of elderlies suffering from cardiovascular problem accompanied by related disease and ACE2 is elevated in HF, too (24).

Although the therapeutic effects of hAMSCs-CM have been reported, the mode of action by which hAMSCs-CM exerts its effects has remained to be understood. The current investigation was innovative in focusing on the mode of action of hAMSCs-CM, in the HF model of male Wistar rats.

We expected that this study would fill a part of the scientific gap in the treatment of fatal HF disorder. A graphical abstract of this study has been presented in Fig. 1.

MATERIALS AND METHODS

The hAMSCs-CM preparation

The amniotic membranes were provided by post-partum donors in Shahid Akbar Abadi Hospital who signed written consent. The amniotic origin of isolated cells was confirmed using fluorescence-activated cell sorting (FACS) like our previous job (25). In short, provided cells were cultivated for 48h in a mixture of α-MEM, containing 10% FBS (Gibco, Australia), 100U/mL penicillin, 2mM L-glutamine, and 100µg/mL streptomycin. Phosphate-buffered saline (PBS) was used to wash the cells 3 times. Then, the medium was swapped with a serum-free α-MEM to harvest CM. The MSCs were placed in an incubator under hypoxic conditions (94%N2, 5%CO2, and 1%O2) for 48h, to yield CM. Finally, samples were centrifuged (1200 rpm) and were filtered through a 0.22 m filter and stored at -80°C.

Animal Models

Forty Wistar male rats(180–230g) were purchased from the Iran University of Medical Sciences and randomly divided into four groups; 1) Control: animals with no treatment, 2) HF: animals received 170mg/kg isoproterenol(Sigma, Aldrich, USA) subcutaneously for four successive days, 3) Culture Media: under deep anesthesia with a mixture of ketamine (80mg/kg) and Xylazine (5mg/kg), HF animals were injected 150µl cell-free DMEM into four points of the myocardium with a 31gauge needle along the left

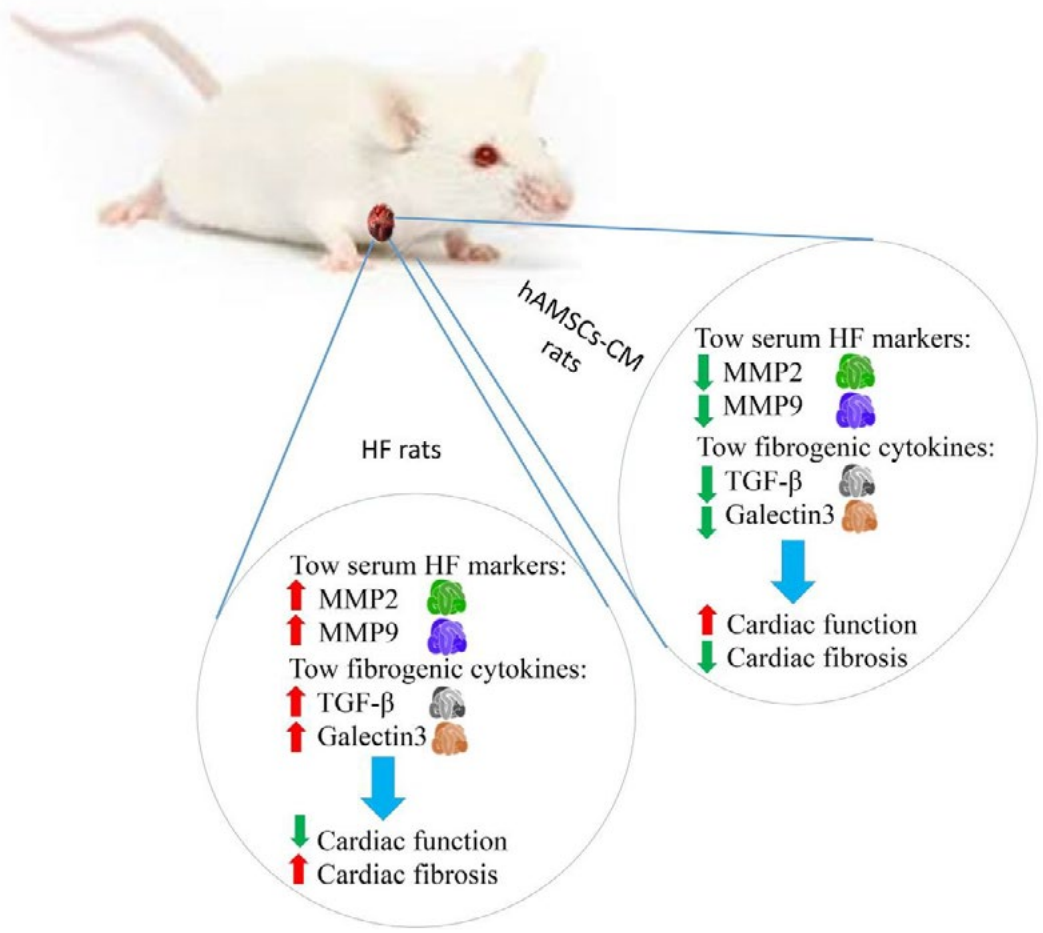

Fig 1. A graphical abstract: heart failure (HF) model rats treated with hAMSCs-CM controlled several factors related to cardiac fibrosis resulted in cardiac function improvement

anterior descending artery four weeks after the last ISO injection, and 4) Conditioned Medium (CM): HF animals were injected 150µl CM with the same condition of Culture Media group.

Echocardiography assessment

After anaesthetizing and shaving the chest and applying an acoustic coupling gel, the echocardiography test was accomplished using a VIVID-7 echocardiography device (GE Vigmed Ultrasound Norway) equipped with a 10s cardiac phase array transverse. A parasternal 2D short-axis view at the level of papillary muscles was selected to record LVDd and LVDs. Then, EF% and FS% were measured.

ELISA assay

After echocardiography, a blood sample was collected and was centrifuged(600 g for 10min at 4^{o}c) to detach serum. The levels of all factors in the serum were assessed using ELISA kits from RayBiotech, Inc. The ELISA plates were then analyzed at a wavelength of 450 nm using an ELISA Reader called Synergy MX BioTek. The results were reported in picograms per millilitre (pg/ml).

STATISTICAL ANALYSIS

All values were presented as mean ± SEM. The one-way analysis of variance and the Tukey test on Prism v5.0 (GraphPad Software, La Jolla, USA) was used for data analysis. P-values less than 0.05 were regarded as significant.

RESULTS

Effects of hAMSCs-CM on cardiac function

Four weeks after CM administration, EF and FS significantly decreased in the HF and Culture Media group compared with the control group ($p<0.001$). EF and FS were significantly improved in CM groups compared with HF groups ($p<0.001$). Although, EF and FS comparison of CM with control revealed a significant difference ($p<0.05$). In other words, treatment with hAMSCs-CM could not return cardiac function up to a normal range (Fig2 and Fig3, A and B).

Effects of hAMSCs-CM on serum level of MMP-2, MMP-4, TIMP, ANG, PRA, and ACE

The ELISA assay revealed that 4 weeks after

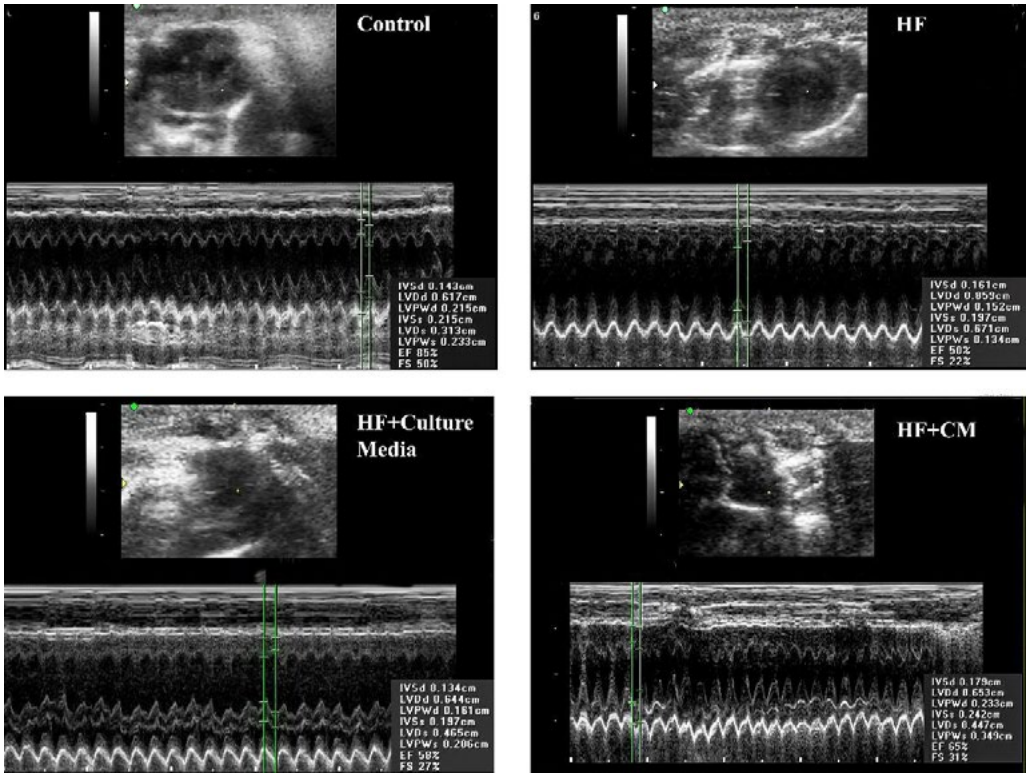

Fig 2. Echocardiographic images of all experimental groups. Ejection fraction (EF) was reached from 50% (HF group) to 65% (CM group) after hAMSCs-CM treatment.

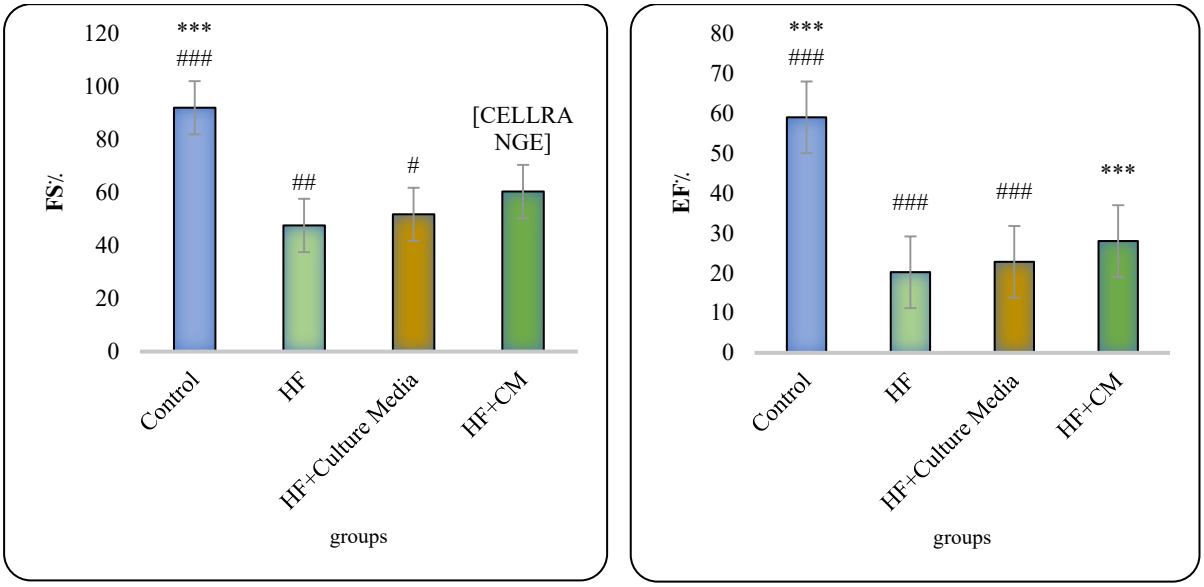

Fig 3. Echocardiographic evaluation of Cardiac function. Cardiac function parameters 4weeks after the intramyocardial administration of hAMSCs-CM(n=10). A) EF% and B) FS%. ###P<0.001, ##P<0.01, #P<0.05 vs. control; ***P<0.001, **P<0.01 vs. HF. Data are presented as mean ± SEM.

intramyocardial delivery of conditioned medium, serum level of MMP2 significantly rose in HF and Culture Media (p<0.01), as well as the CM group (p<0.05) compared with the control. Nevertheless, MMP2 in the CM group significantly declined compared with HF(p<0.01) (Fig5, A).

Serum MMP9 was elevated in HF and culture media(p<0.001), as well as CM group (p<0.05) in comparison with control.

Like MMP2, the level of MMP9 decreased after CM treatment compared with HF (p<0.001) (Fig5, B). CM treatment could not return MMP2 or MMP9 down to a normal range.

The results of the ELISA assay showed that serum

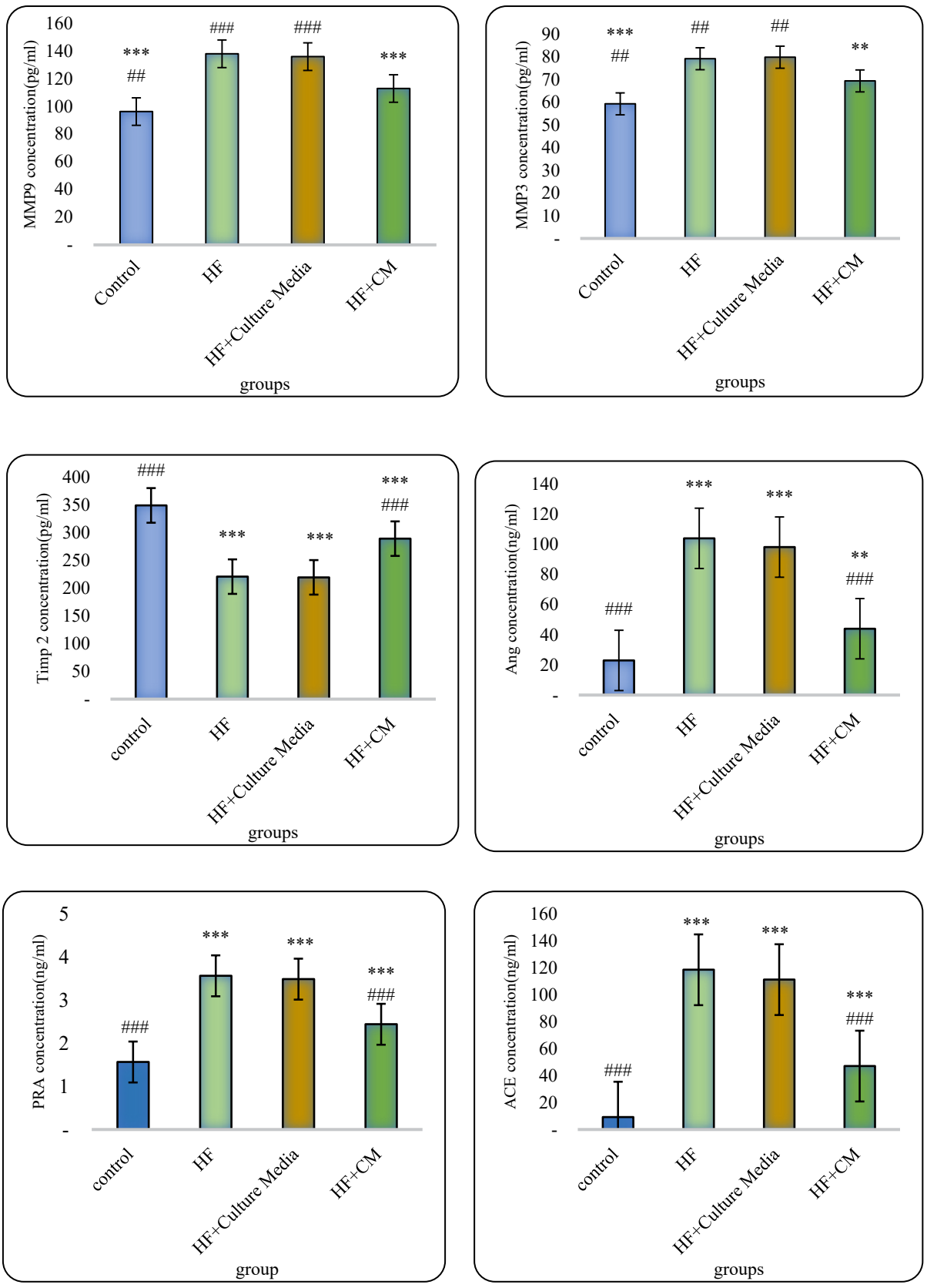

Fig4. ELISA Assay of serum level of A) MMP-2, B) MMP-9, C) TIMP-2, D) ANG, E) PRA, and F) ACE 4weeks after intramyocardial administration of hAMSCs-CM(n=10). ###P<0.001, ##P<0.01, #P<0.05 vs. control; ***P<0.001, **P<0.01 vs. HF. Data are presented as mean ± SEM.

TIMP-2, ANG, PRA, and ACE significantly increased in HF, Culture Media, as well as CM groups compared with the control group ($p<0.001$) while after hAMSCs-CM administration, they were reduced in CM group compare with HF group(all $p<0.001$) but all of them were significantly higher than the controls ($p<0.001$) expect for ANG that was significantly lower than the controls ($p<0.01$)(Fig4, A).

DISCUSSION

Maladaptive cardiac remodeling is the most underlying reason for the death of patients with developing HF. It occurs when an ischemic event triggers the cell death pathways such as apoptosis and necroptosis. The phenomena further cause cardiac fibrosis formation (26).

Fibrotic myocardium impairs cardiac function and eventually, gives rise to HF and even cardiac sudden death. There is a positive correlation between the size of the fibrotic area and developing HF (27, 28). Therefore, preventing cell death and fibrosis formation has emerged as the main point of HF studies (29).

In recent decades, to treat cardiac diseases Mesenchymal Stem cells have been widely used and, hAMSCs have the most tendency to differentiate into cardiomyocytes. Moreover, they are inexpensive, and are easily accessible (8, 10, 30). However, the risk of tumorigenicity and the need to long-term follow up restricted the application of MSCs in clinical trials. As paracrine secretion of MSCs is rich in various growth factors, cytokines, nuclear acids, and therapeutic peptides without the aforementioned drawbacks (9, 11). We hypothesized that hAMSCs-CM could improve HF condition.

Our study provided evidence that administering hAMSCs-CM directly into the heart increased EF and FS. In a study Timmers L et al. reported that hMSCs-CM therapy led to angiogenesis. Their results showed that treatment with hMSCs' secretions increased capillary density, and resulted in a rise in myocardial perfusion which gave rise to decreased infarct area and preserved EF and FS in an I/RI pig model (31). Importantly, reduced infarct size is negatively associated with developing post-MI HF (26, 32).

In the current study, we reported that hAMSCs-CM increased TIMP-2 fibrogenic factor as well as reduced serum levels of MMP-2, MMP-9, ANG, PRA, and ACE.

Our study is consistent with the findings of Daltro and colleagues, who showed that treatment with either BMMSCs or their CM led to a significant decrease in cardiac fibrosis, improvement in cardiac function, and reduction in arrhythmia incidence in a mice model of HFD-induced obesity. However, they stated that only MSCs could reduce the cardiac tissue level of MMP9, and TIMP1 but not the CM, which conveyed that the CM improved cardiac function independent of MMP9. The discrepancy probably is due to different cell lines, animal models, and sampling (1).

Moreover, targeting therenin–angiotensin–aldosterone system (RAAS) significantly increased survival in chronic HF with decreased EF. Activated RAAS leads to chronic HF development via retention of salt and water and systemic vasoconstriction (33). In response to reduced cardiac output it also improves organ perfusion through reforming and maintaining intravascular volume but long-term RAAS activation causes inappropriate ventricular remodeling as well as volume (34). RAAS activation plays a similar role in acute HF(AHF) (35). RAAS is a key system in HF because its inhibition is one of the HF treatments (33).

Furthermore, in a retrospective assessment from the BLAST-AHF trial, overexpressed PRA was related to a higher risk of rehospitalization or mortality during 30 days and baseline PRA was elevated despite more use of aldosterone receptor antagonists. So, PRA activation predicts adverse AHF outcomes. Moreover, PRA may act as an AHF target. However, it is not obvious whether the relation between elevated PRA and adverse AHF outcomes is because of renin-induced overactivation of the RAAS or the increased PRA is because of the greater severity of baseline HF. In a clinical trial, the administration of Aliskiren, as a direct renin inhibitor with the standard AHF therapeutics did not affect rehospitalization or cardiovascular mortality at 6 or 12 months after discharge (36). According to Ueda et al. study, higher PRA is associated with more advanced disease in AHF (37)

Ueda et al divided a cohort study on clinical outcomes of PRA on AHF patients based on average PRA (3.4 ng/mL/h) and reported that patients with higher PRA had greater cardiovascular death during 29 months. In the ASTRONAUT trial, in HF patients with decreased EF who were administered Aliskiren, PRA was reduced early and persistently. However lower baseline PRA was accompanied by better outcomes, Aliskiren treatment did not improve outcomes during one year (38).

Moreover, Chirinos et al. evaluated the clinical and proteomic associates of plasma ACE2 protein in a large cohort of HF people. According to this study, the ACE2 shows carboxypeptidase activity, which leads to ACE-associated AngII production as a result of the AngI degradation to Ang1 to 9 and the AngII degradation decreases its effectiveness. Moreover, the Ang1 to 7 production shows protective effects. Mentioned mechanisms opposed several negative effects of AngII, which is important in pathological situations with the overstimulated RAAS. Ang 1 to 7, shows a kind of biological effects, which are against AngII(23). Furthermore, ACE2 works independently on RAAS to adjust the intestinal microbiome as well as amino acid

homeostasis (39).

CONCLUSION

Our study has yielded valuable insights into the treatment of heart failure, as we have demonstrated that intramyocardial delivery of hAMSCs-CM can reduce cardiac fibrosis induced by ISO. Treatment with hAMSCs-CM also resulted in improved cardiac function and adjusted the serum level of MMP-2, MMP-4, TIMP, ANG, PRA, and ACE. In general, our results pointed out the therapeutic properties of hAMSCs-CM in male rats subjected to ISO-induced HF.

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Summer 2023, Volume 8, Issue 30 (9-16)

Personalized Medicine for HIV Control: A Systematic Review Study

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DOI: 10.22034/pmj.2023.2011747.1015

Submitted: 2023-08-17
Accepted: 2023-09-18

Keywords:
HIV infections
Personalized Medicine
Antiviral inhibitors
Gene-editing approach

Abstract:
There were more than thirty-eight million HIV infections worldwide. Combination antiretroviral therapy (cART) has progressed to the point where invisible viral loads are now feasible, and HIV carriers frequently lead almost everyday lives with considerably greater average life expectancies than in the past. However, there is still no cure for the disease. Even though the ailment usually advances to a chronic state, an individual's unique course of progression may differ significantly from the average and manifest distinctively for each patient. This diversity begs whether a typical treatment strategy is appropriate for a patient.

INTRODUCTION

AIDS is an illness that presents its victims with various issues (1). Human Immunodeficiency Virus (HIV) is the disease's primary causal agent (2). HIV severely harms the immune system. After this virus was discovered in the United States, the illness has transformed throughout the last several decades and is now spreading over the whole globe (3). A kind of T-lymphocyte cell called CD4 is damaged and destroyed by HIV because CD4 cell density in a healthy person's body ranges from 500 to 1500 cells per cubic meter but fewer than 200 in a person with the illness (4, 69). This harm accumulates over time and results in various illnesses in the patient, including cancer (5).

It is possible to mention bodily fluids, including blood, semen, vaginal secretions, and breast milk, as possible transmission routes for this illness(6). Lymph nodes that are enlarged, exhaustion, frequent fevers, headaches and other bodily pains, vomiting and nausea, weight gain, diarrhoea, vaginal and oral infections, pneumonia, and shingles are all signs of the chronic stage of HIV(7). Given that HIV affects a person's DNA, this illness is incurable, and there is no cure for its causes (8). However, efforts have been made to control this illness, and the only effective treatment is the use of antiviral medications that a doctor prescribes (9). If the illness is not controlled, the patient will contract AIDS, and their body will not be able to fight infectious diseases (10). Consequently, it makes a person more susceptible to illnesses like meningitis, oral thrush, and cytomegalovirus (11).

Because there is no treatment for this illness, it is best to follow the adage "prevention is better than cure" and practice good hygiene to avoid contracting HIV(12). For everyone to be aware of this illness and to pay greater attention to their health, the best course of action is to educate the public, particularly teens, about HIV and how this disease is spread. A person-centred medical approach may find the finest answers in this area (13).

Most HIV transmission routes have much to do with lifestyle, personal habits, and behaviors. For instance, using drugs, smoking, or engaging in unhealthful sexual behavior raise the risk of contracting the illness (14). Even though these habits are daily in specific groups and nations, they may be changed with the proper guidance and instruction (15). So, utilizing prescription medication may help to avoid this sickness. Uganda and Thailand are two nations that have succeeded in lowering the pace of the spread of this illness following the principles of personal medicine (16).

Lack of preventive and poor personal hygiene will lead to disorder in society and personal life. Individual health first ensures one's health, then the health of other family members, and lastly, in the second stage, the health of whole communities (17). It should not be overlooked that maintaining personal hygiene and health, including vaccination, altering habits and behaviours, and being aware of the hazards of HIV, may be managed with a personal medical approach (18).

HIV in children

A) nce of HIV in children

Children, who made up about 10% of new HIV infections worldwide and comprised an estimated 3.4 million HIV-positive children under 15 in 2010 (19), have been severely impacted by the HIV pandemic. Since introducing antiretroviral treatment (ART), child survival has considerably risen in resource-rich and resource-limited settings (20). Despite an increase in ART coverage, only around 34% of children under 15 who need treatment in low- and middle-income countries get it, compared to 68% of adults (21).

B) Risk of mother-to-child transmission

Researchers found 288 out of 11,285 kids (2.6%) had HIV-related diagnoses. The majority of children (272, 94.4%) were identified as having HIV by a positive HIV fast antibody test performed after the age of 12 months or by a positive HIV-1 DNA test (22). Only 16 out of 288, or 5.6%, of the youngsters had an HIV diagnosis that was considered severe. Infected with HIV at eight weeks: 0.7% (95% CI: 0.6-0.9) of the enrolling children (23). By the time they were 12 months old, 2.2% (95% CI: 1.9-2.5) of the children had been diagnosed; by the time they were 30 months old, 2.6% (95% CI: 2.3-2.9) (22). The cumulative incidence was 0.8% (95% CI: 0.7-1.0) by age eight weeks, 2.7% (95% CI: 2.4-3.1) by age 12 months, and 5.3% (95% CI: 4.7-5.9) by age 30 months in the weighted analysis, which takes into account unobserved test findings from children lost to follow-up or not tested (22).

C) Growth and development

Even without overt AIDS or wasting, children with PHIV tend to be shorter in height, have lower body weights, and enter puberty later than children without the virus (24). Numerous conditions, including viremia, symptomatic HIV infection, malabsorption, inflammation, mitochondrial toxicity, psychosocial conditions, nutritional deficiencies, aberrant nitrogen balance, and altered growth hormone production or action, are linked to this atypical growth (25). The date of pubertal start (Tanner stage2) was considerably delayed for 2086 PHIV compared to 453 HIV-exposed uninfected children according to research employing two large US longitudinal cohorts between 2000 and 2012. The research also discovered that among PHIV, longer HAART duration was linked with somewhat more normal pubertal onset and that higher VL and lower CD4% were related to more delayed pubertal onset (26). These findings imply that early access to HAART promotes more typical development patterns for PHIV (9). However, there are few findings from the SSA where children are more likely to experience malnutrition and other disorders linked to poor growth (27).

D)Sexual and reproductive health

It has been shown in studies that having an STI increases the risk of HIV acquisition and transmission (28), but it is less clear if those with HIV who are on HAART and have a well-controlled HIV infection are more at risk for STIs (29). There is less research on PHIV, despite several studies showing the significant incidence of STIs among adolescents and young adults who are HIV-positive by behavior (30). In comparison to matched, uninfected controls, a study of 638 PHIV-positive teenage girls in the PACTG 219C cohort found higher rates of condylomas acuminate, trichomoniasis, and cervical abnormalities, such as atypical cells, low-grade, squamous intraepithelial lesions, and

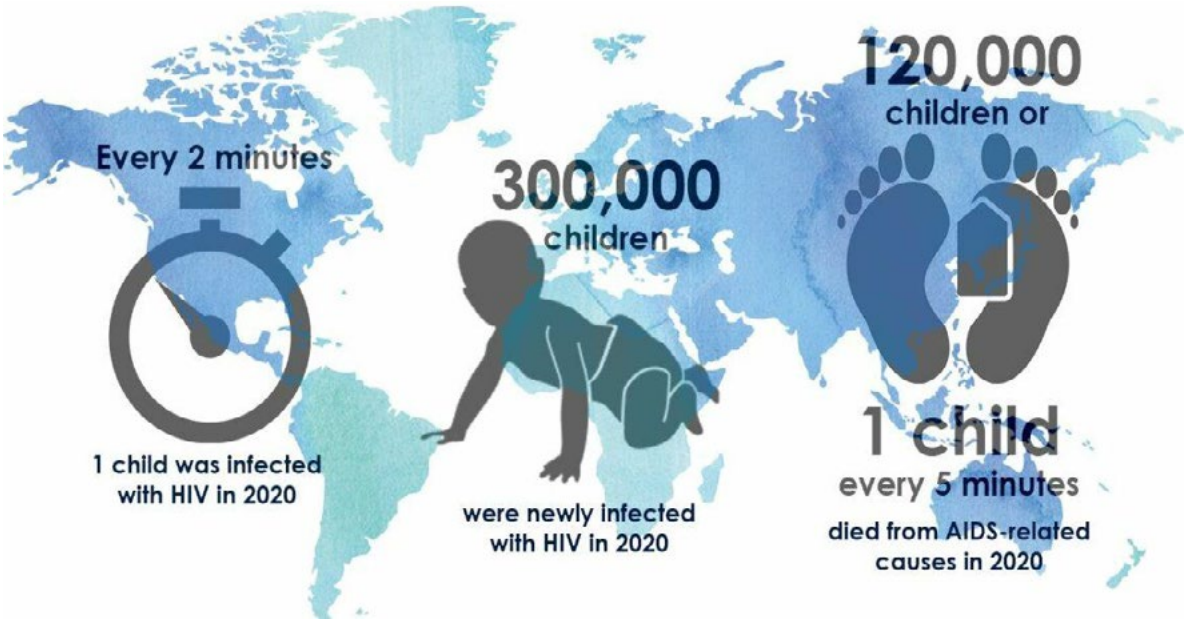

Fig 1. At least one child globally was infected with HIV every two minutes in 2020. (69)

high-grade squamous intraepithelial lesions (26). Compared to the general population, both groups of PHIV and behaviorally infected women in the United States had higher rates of pregnancy and premature births. PHIV women were significantly more likely to elect a pregnancy termination (26). In comparison to uninfected infants born to non-perinatally HIV-infected mothers, uninfected infants born to PHIV mothers were significantly shorter throughout the first year of life (after adjusting for confounding), according to a recent retrospective cohort study of 152 pregnancies in the United States (26). However, the significance of these findings is unclear. Only a small amount of data is available to guide sexual and reproductive therapies in PHIV (31). In a recent systematic analysis, it was determined that women's outcomes, such as the prevalence of HIV and STIs, the use of condoms and other contraceptives, and their retention in care, were better when sexual and reproductive health services were linked with HIV/AIDS services (32). As more people with HIV approach adolescence and adulthood, it is essential to look at more efficient service delivery systems for sexual and reproductive health care (33). Additionally, the seldom acknowledged problem of the sexual and reproductive health of PHIV-men must be addressed (26).

Antiviral inhibitors drug resistance in HIV

Human immunodeficiency virus type 1 (HIV-1) was first identified as the source of the HIV/AIDS epidemic in the early 1980s (34). Approximately 33 million people have perished from the illness in the last 40 years. The three viral enzymes, protease (PR), reverse transcriptase (RT), and integrase (IN), as well as various phases of the viral lifecycle, have all been the subject of multiple antiretroviral medication developments (35). The World Health Organization advises using these antiviral medications in combination with treatment because of their significant efficacy (35). Pre-exposure prophylaxis depends on using RT and IN inhibitors without an HIV vaccine. Treatment for HIV/AIDS relies heavily on antiviral medicines that specifically target the retroviral protease of the human immunodeficiency virus (HIV) (36). This therapy's main drawback is developing antiviral medication resistance, which affects many treated patients and builds up throughout treatment (37).

Types of drug resistance in HIV

A) Transmitted drug resistance

HIV drug-resistant strains may spread from patient to patient, causing newly infected individuals to carry drug-resistant viruses even if they have not yet started antiretroviral therapy. This is referred to as transferred medication resistance and poses a severe risk to the transmission of HIV (38, 62, 63).

B)Acquired drug resistance during antiretroviral treatment

Patients receiving antiretroviral therapy (ART) often see a gradual rise in acquired drug resistance over time (39).

C) Multi-class drug resistance

When a virus develops resistance to one medicine and then develops resistance to another drug from a different class, this phenomenon is known as multi-class drug resistance. Although it is theoretically feasible for a virus to acquire many medication-resistance mutations concurrently, the facts indicate that this is uncommon (40).

D) Resistance to the newer drugs

NRTIs, NNRTIs, and PIs were the only three main medication classes initially available for the treatment of HIV (41). However, the significant cross-resistance across these classes made it unlikely for another NNRTI to be helpful if a patient did not react to one (38). Elvitegravir and raltegravir are examples of integrase strand transfer inhibitors (INSTIs), CCR5 antagonists like maraviroc, and fusion inhibitors like enfuvirtide that were released into the market in 2003(42). There might also be wide genetic variations in drug resistance for the new medication classes. For instance, raltegravir and elvitegravir may resist single mutations, whereas newer integrase inhibitors like DTG and MK-2048 may resist numerous mutations (38). To assess how susceptible HIV is to various antiretroviral medications, resistance tests have been created. There are now two different kinds of tests: genotypic tests, which look for resistance mutations, and phenotypic tests, which gauge a virus's sensitivity to different medications in tissue-culture systems (43, 72).

Phenotypic resistance tests examine viruses' susceptibility to various medications in vitro, and the findings may be very instructive in a research context. However, because of its intricacy, expense, and time commitment (requiring more than a week to complete), this sort of assay is not appropriate for routine clinical testing. Genotypic resistance testing is an alternate strategy that entails sequencing the relevant viral genome segments and analyzing the sequence in light of the virus›s resistance phenotype (44, 64-66).

Forecasting the establishment of medication resistance requires knowing how HIV replicates during treatment (45). HIV infection that persists after treatment indicates the chance that the virus may continue to spread actively and lead to new mutations and medication resistance. On the other hand, the likelihood of new drug-resistance mutations arising from long-lived, chronically infected reservoirs is significantly lowered if treatment suppression successfully stops the infection from spreading (46).

Human immunodeficiency virus (HIV) presents

Table 1. Multi-class drug for HIV treatment.

Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) [61]	Non-nucleoside reverse transcriptase inhibitors (NNRTIs) [62]	Protease inhibitor (PI) [63]	Integrase inhibitors [64]	Post-binding inhibitor or monoclonal antibody [65]	Drugs based on integrase strand transfer inhibitor (INSTI) [66]	Drugs based on nucleoside/nucleotide reverse transcriptase inhibitor (NRTI) [67]
Abacavir	Cabotegravir	Atazanavir	Cobategravir and Rilpivirine	Atazanavir + Cobicistat	Bictegravir + Tenofovir Alafenamide + Emtricitabine	Abacavir + Lamivudine
didanosine	Delavirdine	Darunavir	Cabotegravir	Darunavir + Cobicistat	Dolutegravir + Aabacavir + lamivudine	Abacavir + Lamivudine + Zidovudine
Emtricitabine	Doravirine	Fosamprenavir	Dolutegravir	Elvitegravir + TDF + FTC + Cobicistat	Dolutegravir + Rilpivirine	Tenofovir Alafenamide + Emtricitabine
lamivudine	Efavirenz	Indinavir	Elvitegravir	Elvitegravir + TAF + FTC + Cobicistat	Dolutegravir + lamivudine	Tenofovir disoproxil Fumarate + Emtricitabine
Stavudine	Etravirine	lopinavir + ritonavir	Raltegravir	---	Elvitgravir + Cobicistat + Tenofovir Alafenamide + Emtricitabine	Tenofovir disoproxil Ffumarate + lamivudine
Tenofovir alafenamide	Nevirapine	Nelfinavir	---	---	Elvetgravir + Cobicistat + Tenofovir Disoproxil Fumarate + Emtricitabine	Zidovudine + Lamivudine
Tenofovir disoproxil fumarate	Rilpivirin	Ritonavir	---	---	---	---
Zidovudine	---	Saquniavir	---	---	---	---
	---	Tipanavir	---	---	---	---

one of the highest evolutionary rates ever detected, and a combination of antiretroviral therapy is needed to overcome the plasticity of the virus population and control viral replication (47, 71). Conventional treatments cannot clear the latent reservoir, which remains the major obstacle towards a cure. Novel strategies, such as CRISPR/Cas9 gRNA-based genome editing, can permanently disrupt the HIV genome. However, HIV genome editing may accelerate viral escape, questioning the approach's feasibility (48). Here, we demonstrate that CRISPR/Cas9 targeting single HIV loci only partially inhibits HIV replication and facilitates rapid viral escape at the target site (49). A combinatorial approach of two strong gRNAs targeting different regions of the HIV genome can completely abrogate viral replication and prevent viral escape (50). Our data shows that the accelerating effect of gene editing on viral escape can be overcome. As such, gene editing may provide a future alternative to control HIV infection (51).

Efficient targeting and editing of HIV by CRISPR/Cas9 The researchers evaluated the ability of stably expressed gRNAs to target and edit HIV DNA. Two gRNA sequences designed to target the HIV-1 LTR region were expressed in a lentiviral vector with Cas9 endonuclease (52). GRNAs were intended to target the viral structural matrix protein, protease, reverse transcriptase, and integrase, all essential for virus replication (53). The researchers infected Jurkat cells containing a nearly complete copy of HIV with gRNA-containing lentiviruses that target the LTR region, the matrix structural protein, or the integrase enzyme (54). Deep sequence analysis revealed specific genome editing events at the target site in 100%, 76%, and 90.1% of the sequences for LTR6, MA3, and IN5 gRNAs, respectively (47). Therefore, the researchers focused on the LTR region and selected two gRNAs, LTR4 and LTR6, which target the SPI binding region and the TAR loop (47, 70).

Gene Editing of HIV-1 Co-receptors to Prevent and/or Cure Virus Infection

Functional or sterilizing treatment can be achieved using gene editing technologies, which show promise both in vitro and in vivo. To be a successful treatment, gene editing efficiency needs to be increased. Successful gene editing technologies are a desirable alternative for HIV-1 therapy of the future due to their potential

advantages (55). All gene editing approaches must overcome obstacles before they can be developed into an appealing curative HIV-1 therapy. Any gene editing technique to combat HIV-1 will also face difficulties detecting and altering cells at various anatomical areas or altering precursor cells that eventually go to tissue sites. For any in vivo gene editing approach, a delivery system that can be transported to multiple locations will be highly beneficial (56). It is unclear if tissue-resident cells have been effectively changed using gene editing of HIV co-receptors in vivo. Nevertheless, the ability to engraft into various tissue compartments has been demonstrated when hematopoietic stem/progenitor cells are edited with a ZFN targeting CCR5(57). Infected NHPs' guts may be replenished with virus-repleted CD4 central memory T cells using these modified cells. The peripheral blood reservoir and all latent viral reservoirs are anticipated unaffected by co-receptor editing for HIV-1 infection. For instance, it would be less likely to target tissue-resident cells successfully (58). Absent a greater knowledge of the mechanism underlying the "Cure" of the "Berlin Patient," ablation of the CCR5 receptor in CD4 T cells has come to dominate research in this field (59). Delivering Cas9/sgRNA ribonucleoproteins directly to infected cells rather than plasmids has reduced off-target effects. In a recent study, R691A SpCas9 mutant delivery using human HSPCs revealed negligible off-target editing while maintaining excellent on-target activity (60). Human CD4 T cells in vitro CXCR4 expression was interfered with using Cas9 RNPs (61, 67, 68).

CONCLUSION

According to the current research, despite being at the center of the arena—providing the stage of concern its raison —people being treated for HIV were both involved by others and marginalized. Members of professional organizations whose specialized professional interests are prioritized in the field should not overlook patients' concerns. In addition to the particular aims of the communities engaged thus far, the overarching goal of guaranteeing patients' survival must be made more tangible and enriched by patients' perspectives: What do HIV-positive patients require today? How might currently existing support networks, such as DAH, help articulate and formulate such requirements during higher-level decision-making processes on appropriate therapies and developing new tools? Single-pill regimens can assure the continued existence of most HIV-positive persons without problems. Is it the proper path to create new and more precise HIV TOS, such as NGS-based HIV TOS, which might improve digitalization and deeper analysis of patient data? Or may other activities that prioritize patients' health(care) requirements be more beneficial to the health and well-being of HIV-positive people? We encourage participatory programs that incorporate all stakeholders and a diverse range of HIV-positive persons to address the question of which HIV TOS improvements should be prioritized.

Acknowledgements

The authors would like to thank the Biotechnology Research Centre staff members of the Parsian BioProducts Company (PBP) in Iran. This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data generated or analyzed during this study are included in this article.

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http://pmjournal.ir Original Article

Summer 2023, Volume 8, Issue 30 (17- 24)

Evaluation of the Low-Frequency Electromagnetic Fields on Biochemical Parameters in the Absence and Presence of Vitamin C in Mice

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DOI: 10.22034/pmj.2023.2011493.1014

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Submitted: 2023-08-13
Accepted: 2023-09-18

Keywords:
Electromagnetic fields
Biochemical parameters
LF-EMF
Total protein levels
Vitamin C

Abstract:
Exposure to low-frequency electromagnetic fields (LF-EMF) has been considered a global concern because of its harmful effects on human health (cancer, neurodegenerative disorders, etc.). According to the International Agency for Research on Cancer, EMF has been classified as a possible cancerous element for human health. Antioxidants such as vitamin C improve the damage caused by EMF by reducing oxidative stress. To evaluate the effects of EMF on the serum total protein, blood sugar, albumin and triglyceride, and the inhibitory role of vitamin C, 40 male BALB/c mice were recruited. Participants were randomly distributed into four groups 1- exposure to LF-EMF, 2- exposure to LF-EMF which received vitamin C (50 mg/kg), 3- exposure to LF-EMF which received vitamin C (100 mg/kg), and 4- control group (no exposure). The experimental groups (1-3) received LF-EMF (50 Hz, 4 mT, 4 hours/day, and 1 month) while both groups 2 and 3 had intraperitoneally injected vitamin C (50 mg/kg, 100 mg/kg) every other day basis respectively. The obtained results demonstrated higher triglyceride and total protein levels and lower albumin and blood sugar levels in the LF-EMF group compared to controls while vitamin C restricts their alterations ($p<0.05$). To sum it up, our data show that intraperitoneal injection of vitamin C restricts the effects of LF-EMF exposure on the biochemical parameters in mice. However, the antioxidant characteristics of vitamin C may be probably involved in the LF-EMF effects of biochemical parameters in mice.

INTRODUCTION

Nowadays, the development of electrical power stations, high-voltage electrical lines, and modern communication devices as well as universe electromagnetic fields precipitously encircled human life. Moreover, man-made electromagnetic fields such as TV, radio, MRI medical instruments, etc. have largely laden our environment. Electromagnetic fields which exist around the electricity supplies, transferring lines and electricity generators are categorized into three main ranges: low-frequency electromagnetic fields (EMF) (50-60 Hz), intermediate frequency (300 Hz-<10 MHz), and radiofrequency range (10 MHz-300 GHz) (1, 2).

The effects of low-frequency electromagnetic fields (LF-EMF) and their biological consequence on human life have attracted researchers globally. Industrialization in developed and developing countries increased the electricity power stations resulting in the widespread concern about the possibility of harmful effects of LF-EMF on human health. Several epidemiologic studies uncovered the deleterious effects of LF-EMF on humans in recent years (3, 4). According to the Wertheimer report, there was a severe correlation between the children's blood cancer and LF-EMF exposure in Denver. He asserted that the higher risk of childhood leukemia at the residential with higher LF-EMF (5, 6). After that, *Savitz et al.* released a publication that supported the Wertheimer report (7). Moreover, researchers published a paper about the association of LF-EMF and various disorders such as suicide (8), cancer (9, 10), and neurodegenerative disorders such as amyotrophic lateral sclerosis and Alzheimer's disease (11).

A solid mass of investigations about the effects of LF-EMF was accomplished on the cellular components including the central nervous system (CNS), genetic material, and proteome and embryogenesis (12, 13). In

the short-term exposure, all and altogether do not show significant deleterious effects on physiological and behavioral parameters. Nevertheless, the long-term animal studies need more investigation (14).

Low-frequency electromagnetic field has been described as electromagnetic oscillating waves having below 50-60 Hz frequency with widespread usage in the world. Despite mass investigations conducted on LF-EMF, it seems that further studies are needed to evaluate the effects of LF-EMF exposure on pathophysiological aspects of human health. Based on the *McCann et al.* the exposure to LF-EMFs have not the critical effects on prokaryotes and eukaryotes (15, 16). However, the report of *Sabine et al.* showed the toxicity of LF-EMF (17) and *Feychting et al.* displayed a significant correlation between exposure to LF-EMF and the incidence of childhood cancer (14).

Some reactive compounds such as free radicals, superoxide anions, hydrogen peroxide and hydroxyl radicals are generated during the various metabolic pathways as a by-product. As well as the reactive oxygen species (ROS) are involved in cellular signalling transduction and gene expression. They are usually involved in determining cell growth, anti-inflammatory response, cellular proliferation and differentiation, and oxidative stress response (18). The free-generated radicals and ROS concentration are balanced by the regulation of the rate of production and clearance. The enzymatic and non-enzymatic antioxidants which provide antioxidant tolerance controlled these procedures to maintain the regular redox hemostatic condition (19). Excessive generation of free radicals and ROS causes the imbalance between oxidants and antioxidants in favor of the former which is called oxidative stress (20).

In the represented study, we aimed to show the effects of LF-EMF on biochemical parameters such as blood sugar, triglyceride, total protein and albumin in Mice whereas the role of vitamin C was investigated consequently.

METHODS AND MATERIALS

Animals

Our study was conducted on 40 healthy male BALB/c mice (25-30 gr) which were purchased from the animal room of Razi Institute (Mashhad, Iran). The participants were freely allowed access to fresh tap water and commercial standardized pelleted food. Relative humidity and ambient temperature of the animal room were 65±5% and 25±2^{OC} respectively. They were kept under 12 light-12 dark cycle and all experiments were performed under the animal experimental care approval (21).

Groups

Mice were randomly divided into four groups: 1- LF-EMF that were exposed to low-frequency electromagnetic fields (50 Hz, 4mT, 4 hours/day, 1 month) (n=10), 2- LF-EMF+50 mg/kg/ vitamin C every other day basis (n=10), 3- LF-EMF+100 mg/kg/ vitamin C every-other-day (n=10) and 4- healthy controls (n=10). Animals received vitamin C by Intraperitoneal injection by other basic after exposure to LF-EMF. Our LF-EMF exposure tool generates electromagnetic wave (50 Hz, 4 mT) that consists of a plastic chamber with a copper line coil that the participants were placed in the chamber with the ability to move restriction-free. The controls however were placed in the same condition and similar intervals while there was no exposure to LF-EMF for them.

Measurements of biochemical parameters

At the end of the study period, participants were anaesthetized and 5 ml cardiac blood was collected for determination of biochemical parameters. Samples were centrifuged for 10 minutes at 1800 g and obtained sera were kept at -20^{oC}. The blood serum was processed in a Mindray SAL-6000 Chemistry & Immunoassay Integrated System (Shenzhen Mindray Bio-Medical Electronics Co) for analysis of the following parameters: total protein (TP), triglycerides (22), cholesterol (CHL), and glucose (GLC). The biochemical kits and calibration controls used were acquired from Lab-test Diagnosis (Lagoa Santa, Minas Gerais, Brazil), and were used according to protocols established by the manufacturer.

STATISTICAL ANALYSIS

The mean values and standard deviations were analyzed for obtained data by SPSS software (SPSS 16.0, SPSS Inc., Chicago, IL, USA). The data normality was evaluated by the K-S test; however, the One Way ANOVA statistical test was used to comparison of the obtained results between controls and EMF. The $p<0.05$ was considered as the statistically significant difference.

RESULTS

Total protein

The level of total protein in the LF-EMF exposed group (5.34 ± 0.19 mg/l) was significantly raised in comparison to the controls (4.60 ± 0.08 mg/l) ($p<0.001$). The data demonstrated that the level of total protein in LF-EMF which received vitamin C (50 and 100 mg/kg) were 5.08 ± 0.12 mg/l ($p<0.001$) and 4.44 ± 0.07 ($p<0.001$) that are lower than LF-EMF group respectively (Table 1) (Figure 1).

Albumin

The mean level of albumin in the serum of controls was 3.10 ± 0.06 mg/l but it decreased in the LF-EMF group to 2.48 ± 0.04 which showed a statistically significant

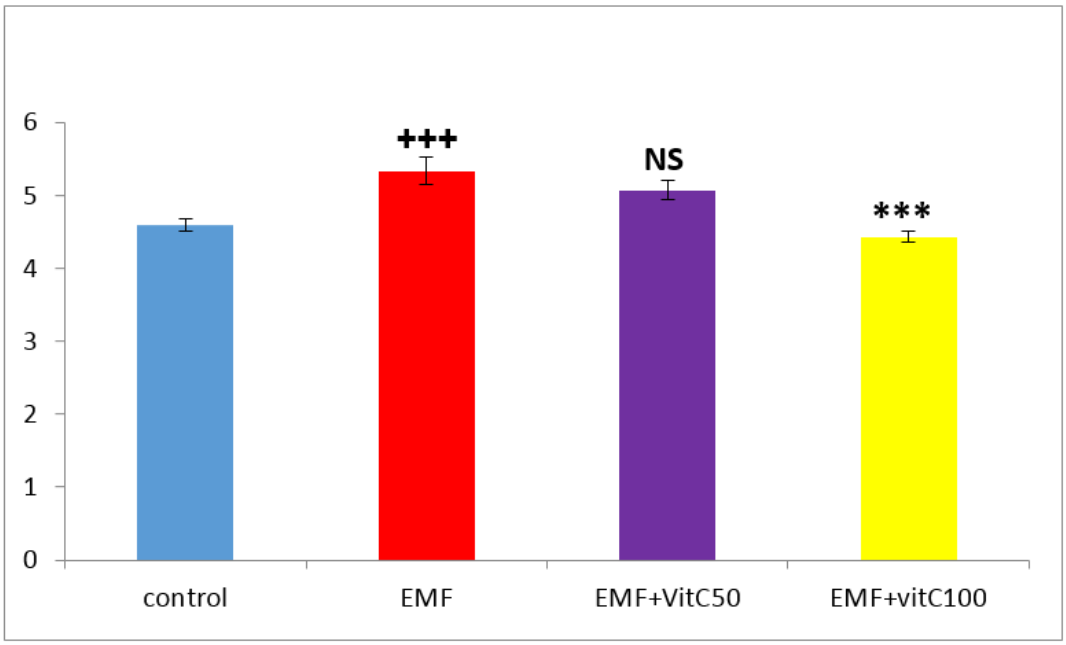

Fig 1. Figure1: Evaluation of total protein level in participants
+++: significant difference between controls and EMF group (p<0.001)
***: significant difference between EMF and EMF+vitamin C (100mg/kg/any other day) group (p<0.001)
NS: Non significant correlation

Table 1. Serum biochemical parameters in control and different exposed groups after 1 month exposure to low-frequency electromagnetic fields

Biochemical parameters	Control	LF-EMF	LF-EMF+50 mg/kg vitamin C	LF-EMF+100 mg/kg vitamin C
Total protein mg/l	4.60 ± 0.08	5.34 ± 0.19	5.08 ± 0.12	4.44 ± 0.07
Albumin mg/l	3.10 ± 0.06	2.48 ± 0.04	2.80 ± 0.08	2.84 ± 0.04
Blood sugar (Glucose) mg/100 cc	250.4 ± 12.6	178 ± 5.3	223 ± 6.5	234 ± 23.6

difference between the two groups (p<0.001). Moreover, the level of albumin in LF-EMF exposed group received 50 and 100 mg/kg vitamin C (2.80 ± 0.08) and (2.89 ± 0.03) were significantly higher than LF-EMF exposed group (p<0.01) (Table 1) (Figure 2).

Triglyceride

The level of triglyceride in controls was 46.6 ± 2.9 mg/dl while it raised to 79.6 ± 9.3 in LF-EMF exposed group (p<0.001). The levels of triglyceride in the LF-EMF exposed group received 50 and 100 mg/kg vitamin C were 53.8 ± 3.3 and 42.6 ± 4.1 which significantly decreased in comparison to the LF-EMF exposed group (p<0.01 and p<0.001) (Table 2) (Figure 3).

Cholesterol

The level of Cholesterol in controls, LF-EMF, LF-EMF received 50 and 100 mg/kg vitamin C were 119.4 ± 5.8, 119.8 ± 4.8, 115.2 ± 9.3, and 114.4 ± 4.6 mg/dl respectively which demonstrated no statistically significant different among them (Table 2) (Figure 4).

Blood sugar (Glucose)

Our obtained results uncovered that the level of glucose in controls was 250.4 ± 12.6 mg/dl but it reduced significantly in the LF-EMF exposed group to 178 ± 5.3 mg/dl (p<0.01). The mean of serum glucose in LF-EMF received 50 mg/kg vitamin C was 223 ± 6.5 mg/dl showing the no significant difference, while it was raised to 234.4 ± 23.6 in LF-EMF received 100 mg/kg vitamin C showed a statistically significant difference with LF-EMF exposed group (p<0.05) (Table 1) (Figure 5).

Liver weight

The mean range of lever weight in controls, LF-EMF, LF-EMF received 50 and 100 mg/kg vitamin C were 0.049 ± 0.004, 0.055 ± 0.002, 0.052 ± 0.003, and 0.037 ± 0.008 gr respectively. According to our data, the weight of the liver in the LF-EMF received 100 mg/kg vitamin C was significantly lower than that of the LF-EMF exposed group (p<0.05) (Table 3) (Figure 6).

DISCUSSION

Vitamin C is known as an important antioxidant that plays a role as a cofactor in many enzymatic reactions during infections and inflammation and also protects cells from various oxidative damages.

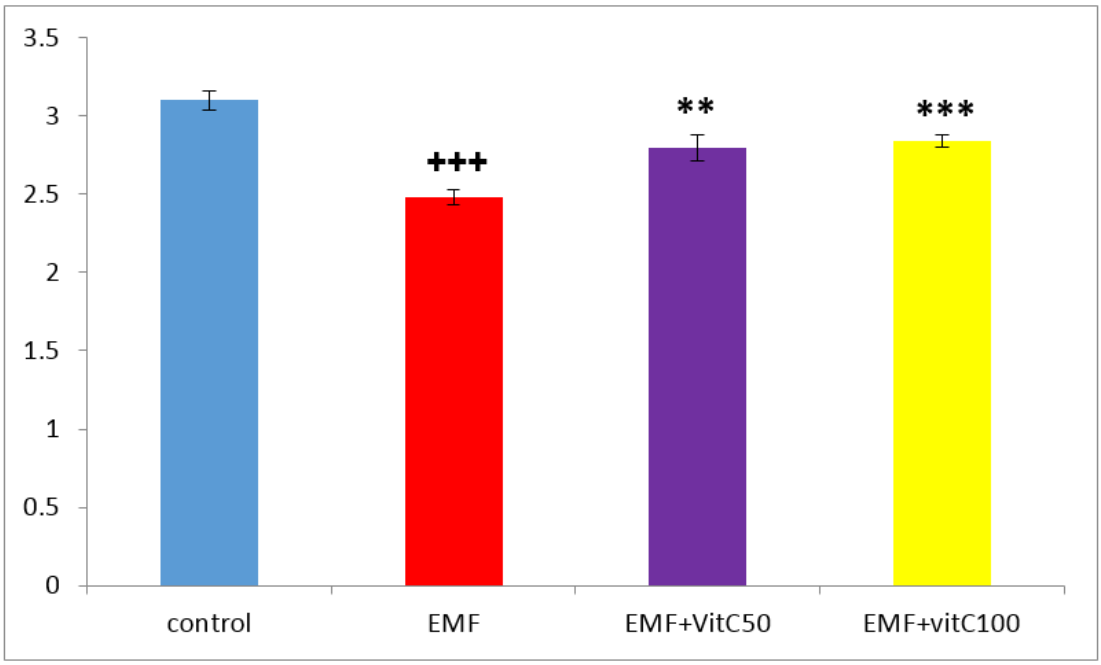

Fig2. Figure 2: Evaluation of the level of Albumin in participants
+++: significant difference between controls and EMF group (p<0.001)
***: significant difference between EMF and EMF+ vitamin C (100mg/kg/any other day) group (p<0.001)
**: significant difference between EMF and EMF+ vitamin C (50mg/kg/any other day) group (p<0.01)

Table 2. The level of triglyceride and cholesterol in serum of control and different low-frequency electromagnetic fields exposed groups after 1 month exposure

Lipid parameters	Control	LF-EMF	LF-EMF+50 mg/kg vitamin C	LF-EMF+100 mg/kg vitamin C
Triglyceride mg/100 cc	46.6 ± 2.9	79.6 ± 9.3	53.8 ± 3.3	42.6 ± 4.1
Cholesterol mg/100 cc	119.4 ± 5.8	119.8 ± 4.8	115.2 ± 9.3	107.6 ± 9.1

Vitamin C plays an important role in reducing the risk of diseases such as cancer, Alzheimer's, Parkinson's and other degenerative diseases (22). The various effects of electromagnetic fields on living cells are a complicated phenomenon involved in the different metabolic pathways (23). These effects have been initiated by physicochemical reactions such as the polarization of electrolytes and structural biomolecules leading to the generation of ROS and free radicals, weakening covalent bonds, hydration alteration, and change of dipoles' spin which consequently affects the biochemical parameters (24-26). In the present study, exposure to low-frequency electromagnetic fields (LF-EMFs) altered the biochemical parameters in mice serum in the presence and absence of vitamin C. Accordingly, the level of total protein, albumin, triglyceride, and blood glucose along with the liver weight were significantly affected among participants.
In the current study, the level of total protein was raised significantly in the LF-EMF exposed group whereas the level of albumin was reduced which may demonstrate the higher level of immunoglobulin generation in participants exposed to LF-EMF. In other words, it can be inferred that exposure to LF-EMF increased the level of antibodies, inflammatory and pro-inflammatory proteins, homeostatic and fibrinolytic polypeptides and proteolytic modulators (27). In other words, exposure to electromagnetic fields not only increased the generation of ROS and free radicals in the liver and other tissues, but it also led to a significant alteration in the level of antioxidants in plasma that showed oxidative stress (28). Based on our investigations, LF-EMF may be involved in oxidative stress-related biomolecules and/or tissue impairments which activated the immune responses and raised the level of total protein in our study. Determination of albumin level demonstrated a significant reduction in the LF-EMF exposed group, demonstrating the severe hepatic problem. The liver is the unique source of albumin production. Clinical association of liver damage is consequently mirrored in albumin concentration which is associated with severity of impairment (29).
Our results showed that the intraperitoneal administration of vitamin C increased the albumin level in participants reduced by LF-EMF exposure. Thereby, LF-EMF may cause serious hepatic damage that deceased albumin production whereas vitamin

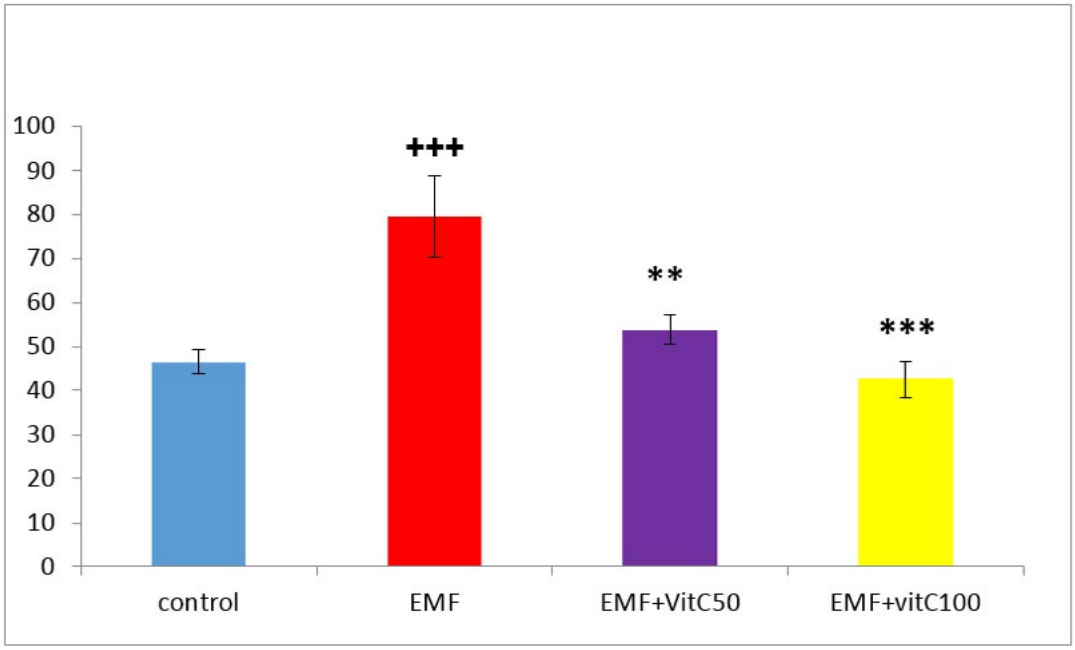

Fig3. Figure 3: Evaluation the level of triglyceride among participants
+++: significant difference between controls and EMF group (p<0.001)
***: significant difference between EMF and EMF+ vitamin C (100mg/kg/any other day) group (p<0.001)
**: significant difference between EMF and EMF+ vitamin C (50mg/kg/any other day) group (p<0.01)

Table 3. The weight of liver in participants after 1 month exposure

Tissue weight	Control	LF-EMF	LF-EMF+50 mg/kg vitamin C	LF-EMF+100 mg/kg vitamin C
Liver (mg)	49 ± 4	55 ± 2	52 ± 3	37 ± 8

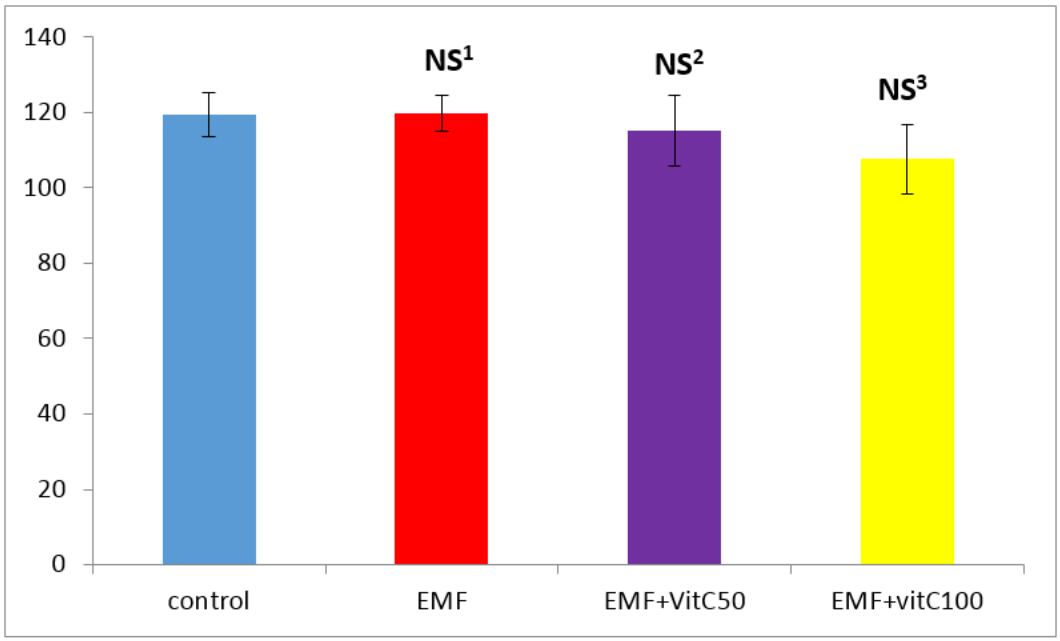

Fig4. Figure 4: Evaluation the level of cholesterol among participants
NS1: No significant difference among control and EMF groups
NS2: No significant difference among EMF and EMF+ vitamin C (50mg/kg/any other day) groups
NS3: No significant difference among EMF and EMF+ vitamin C (100mg/kg/any other day) groups

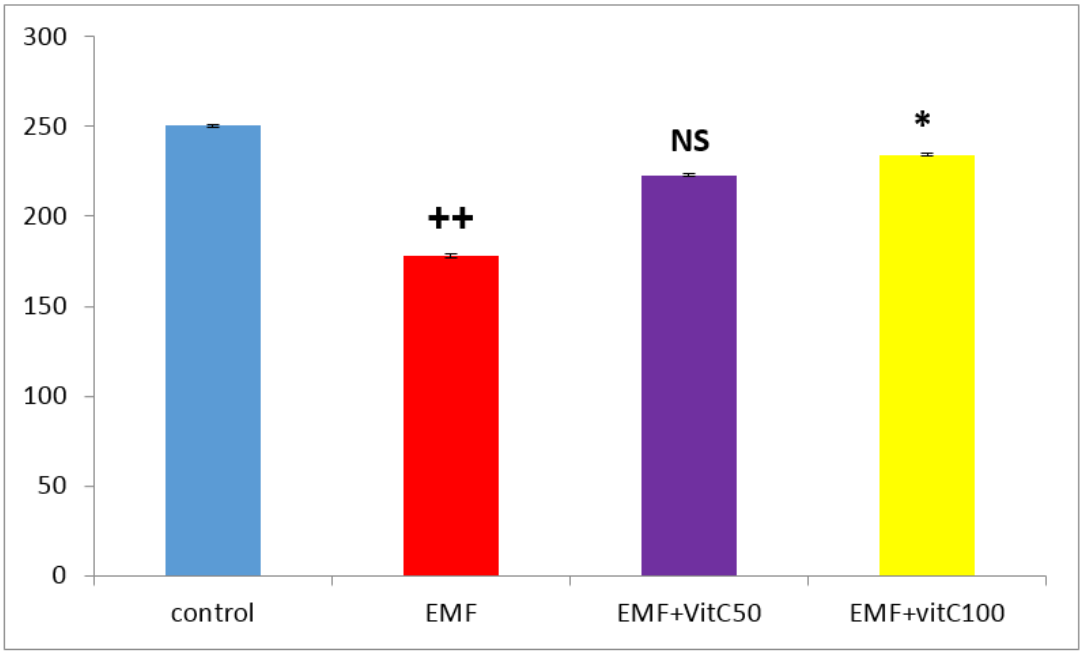

Fig5. Evaluation the level of glucose among participants
++: significant difference between controls and EMF group ($p<0.01$)
*: significant difference between EMF and EMF+ vitamin C (100mg/kg/any other day) group ($p<0.05$)
NS: significant difference between EMF and EMF+ vitamin C (50mg/kg/any other day) group

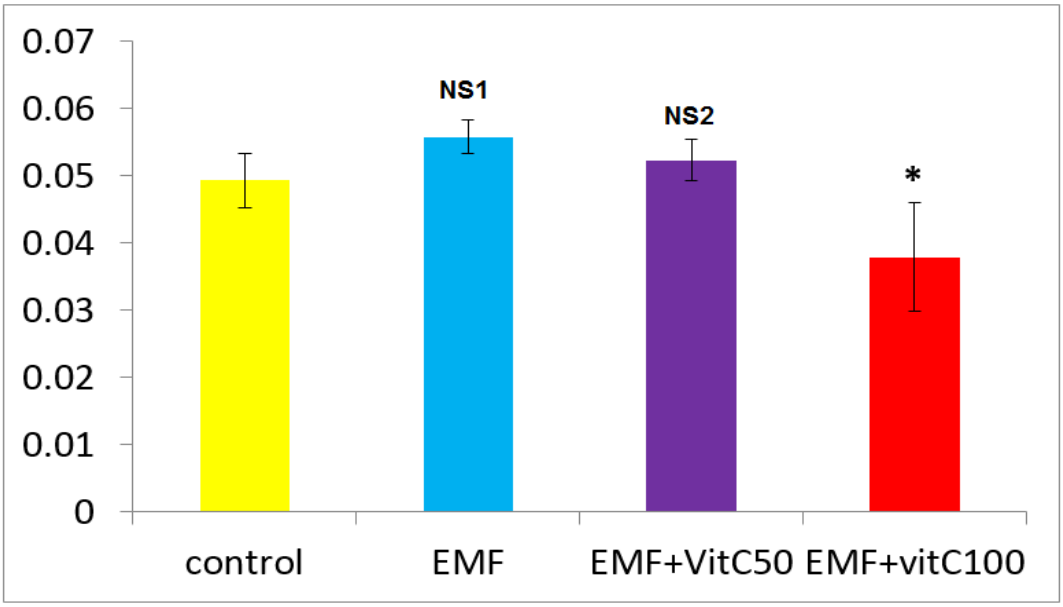

Fig6. Figure 6: Evaluation the weight of liver among participants
*: significant difference between EMF and EMF+ vitamin C (100mg/kg/any other day) group ($p<0.05$)
NS1: no significant difference between EMF and control group
NS2: no significant difference between EMF and EMF+ vitamin C (50mg/kg/any other day) group

C administration improved albumin generation consequently. These changes are considering the serious pathological responses correlated to oxidative stress represented due to LF-EMF exposure. The liver weight was increased significantly demonstrating a higher amount of inflammation and consequently higher blood circulation in the liver in comparison to the LF-EMF exposed group received 100 mg/kg vitamin C.

Obtained data demonstrate that the level of triglyceride and Cholesterol were increased while the level of glucose decreased in the LF-EMF exposed group. It

may show that one month of exposure to LF-EMF (50 Hz, 4 mT) caused gradual metabolic changes with reduced levels of total protein and glucose in the participants when compared to controls (30). However, the pathological effects of oxidative stress were preceded more logically. Intraperitoneal administration of vitamin C in exposed mice improved the level of biochemical parameters.

Exposure to an electromagnetic field (50 Hz) can cause oxidative stress and stimulate the secretion of mineralocorticoids (such as cortisol) which increases the glucose level in exposed animals (31). Exposure to EMF affects cellular and/or tissue membranes by changing the ion disturbance, protein, and membrane charges and making dysfunction in bilayer lipid membrane lead to excessive permeability of glucose to cell/tissue (32). Administration of vitamin C with the extremely low standard potential of reduction (280 mV) improves the intracellular/extracellular antioxidant capacity, thereby decreasing lipid peroxidation, free radical generation, and neutralizing ROS (33-35).

CONCLUSION

Striking effects of LF-EMF on biochemical parameters were observed in the exposed mice. We can conclude that the electromagnetic fields can affect the biochemical parameters. Exposure to LF-EMF (50 Hz, 4 mT, 4 hours/day, 1 month) increased the total protein and decreased the albumin level whereas vitamin C regulated the alternation of both parameters. The pathological effects of LF-EMF seem associated with oxidative damage caused by generated free radicals and ROS The antioxidant capacity of vitamin C restricts the LF-EMF damage to the liver, and also regulates the level of biochemical parameters in participants. To investigate the precise effects of LF-EMF exposure on liver damage and the role of pathologic oxidative stress on biochemical parameters further studies are needed.

Acknowledgement

The authors thank the staff and members of the Department of Biology, Islamic Azad University, Mashhad branch. This paper is the result of a part of the M.Sc. tsis of Melika Parsian Mehr.

Funding information

No financial support has been received from any center in the research.

Conflict of interest

The authors reported no conflict of interest in the present study.

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http://pmjournal.ir Review Article

Summer 2023, Volume 8, Issue 30 (25- 31)

Targets for Anti-HIV1- Agents as Personalized HIV Therapy: a Review Study

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DOI: 10.22034/pmj.2023.2011750.1016

Submitted: 2023-08-23
Accepted: 2023-09-19

Keywords:
HIV therapy
Anti-HIV-1 Agents
HIV medication resistance

Abstract:

The enormous genetic variety of the viral population harbored by the patient and the large volume of therapeutic alternatives characterize HIV therapy. Each patient and period has its viral population. The enormous number of therapy possibilities makes selecting an ideal or near-optimal therapy challenging, especially among therapy-experienced patients. Over the last decade, computer-based medication selection that measures viral resistance to pharmaceuticals has become a norm for HIV patients. We explore the qualities of available systems and the field's viewpoints.

INTRODUCTION

HIV is one of the most rapidly changing diseases known, and there is currently no HIV vaccine (1). Because the patient cannot be treated for the virus once infected, therapy aims to inhibit viral replication, alleviate symptoms, and extend life (2, 3). For this goal, more than two dozen distinct antiretroviral medications have been produced in record time for all other illnesses today (4, 5). Drugs inhibit a multitude of phases in the viral replication cycle (6-8). Although a particular medicine therapy can be beneficial for a long time, even years, the virus ultimately evolves into a resistant variety, resulting in therapeutic failure (9). When this occurs, a new treatment combination that effectively addresses the resistance profile displayed by the viral population currently in the patient must be chosen (10). This is a challenging undertaking, but appropriate tools can assist in selecting effective therapeutic alternatives for these individuals (11). This paper summarizes the history and current state of bioinformatics-based resistance analysis and future prospects (12).

HIV medication resistance assessment history

There are two methods for HIV and other viral resistance analyses. Viruses are tested in vitro for sensitivity to various medicines in phenotypic resistance tests (13). This laboratory approach is highly informative in the context of research (14). However, it is unsuitable for clinical routine testing for numerous reasons: the assay is challenging, requiring only a few highly specialized laboratories to do it, it is costly, and it takes a long time (more than a week) (15). Another option is genotypic resistance evaluation, which involves sequencing the relevant sections of the virus genome while analyzing the sequence concerning the virus's resistance phenotype (16). In industrialized nations, genotypic resistance screening is frequently used as a companion diagnosis in HIV therapy (17). The first attempt to analyse genotypic resistance information related to HIV in history was made utilising tables by expert committees that convened regularly (18). They made judgements based on evidence from the literature, laboratory data, and clinical (19). Regular updates to the resultant mutation lists were and continue to be released (20).

The mutation list has improved the efficacy of currently used antiretroviral treatments, but it has two shortcomings: The first is the table's minimal information content (21). A table, in particular, cannot convey relationships between alterations; instead, each mutation functions independently in giving the virus's resistance to the treatment, and neither the epistemic process nor desensitization is considered (22). The emergence of computerized rules-based systems has solved this constraint (23). In effect, they are sets of rules that can assume more sophisticated forms than the rules implied in the mutation tables (24). Consider the rule that says a virus is resistant to medication D if it possesses mutation M1 but not M2 (25). This

describes the virus's desensitization to medication D due to mutation M2 (26). The computer evaluates the relevant region of a viral genome versus all rules in the set in a rules-based system, also known as a resistance algorithm (27). There are numerous widely used systems, including those provided by the Stanford HIV Archive, Rega Institute, and ANRS (28). These techniques form the foundation of computer-based genotypic resistance data interpretation as additional testing for antiretroviral HIV medication selection (29).

HIV-1 Life Cycle Factors as Anti-HIV-1 Agent Targets

The HIV-1 life cycle is comprised of multiple phases, beginning with the adherence of an HIV-1 particle to the host cell membrane, where linkages between the HIV-1 envelope (gp120) and the cell surface CD4 receptor proceed by binding to the chemokine receptors CXCR4 or CCR5 (30). These contacts activate the HIV-1 fusion protein (gp41), producing cell-viral membrane fusion (31). The virion's contents are then released into the cytoplasm, where viral RNA is converted to double-stranded DNA by RNA-dependent DNA polymerase or HIV-1 reverse transcriptase (HIV-1-RT) (32).

Following that, viral DNA is incorporated into the host chromosome (33). Translation and transcription Using the cell's machinery, Gag and Gag-Pol polyproteins are converted into viral proteins and transported to the cell membrane, where virions are assembled, budded, and matured before being released as functional HIV-1 particles (34). In general, anti-HIV-1 medications should target viral or cellular proteins in the HIV-1 life cycle (35). Furthermore, interactions between such small compounds and target proteins should ideally result in HIV-1-specific inhibitory effects with minimal toxicity (36).

Molecular docking of HIV protease Inhibitors

Six authorized anti-HIV medications were chosen for testing (37). Although 3CLpro-2 and 3CLpro-1 have greater binding energies than all HIV protease inhibitor combinations used as positive controls, 3CLpro-2 has lower binding energy for all investigated inhibitors than its sibling 3CLpro-1 (38). This indicates that 3Clpro-2 has more remarkable binding affinities for inhibitors than 3CLpro-1 (39). Indinavir and darunavir have been shown to have a greater binding capacity to 3CLpro-2 than the other HIV protease inhibitors, and their interaction energy values are comparable to those of HIV inhibitors of protease (40).

When examined, the binding energy of the 3CLpro-2-darunavir complex (-10.24 kJ mol 1) is lower than that of its 3CLpro-2 indinavir equivalent (-10.02 kJ mol 1), showing that darunavir likely has a better affinity for 3CLpro-2 than indinavir (41). Because 3Clpro is required for coronavirus replication, the inhibitory action of these substances on 3Clpro-2 suggests they might be used as anti-COVID-19 therapeutic medicines (42).

New insights into the clinically validated antiretroviral targets

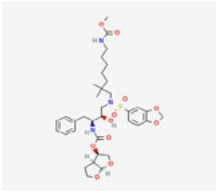
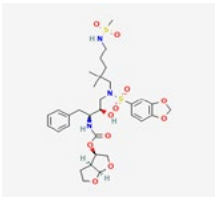
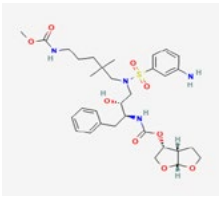
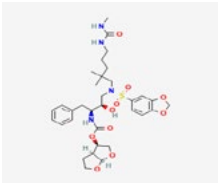
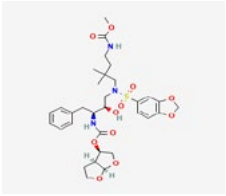
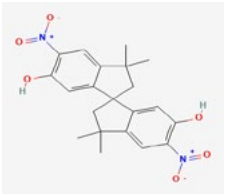
For the clinically validated HIV targets (RT, IN, PR, and CCR5), there is still significant scope for further development of novel inhibitors with distinct mechanisms of action, such as RNase H inhibitors, Nucleotide-competing RT inhibitors (NcRTIs), noncatalytic site (allosteric) IN inhibitors, and PR dimerization inhibitors From the HIV therapy point of view (43), an allosteric inhibitor could restore the potency of an active site inhibitor against multidrug restore the potency of an active, so combined therapy with an active site inhibitor and an allosteric inhibitor may be available as a new anti-HIV strategy to overcome drug resistance (44).

Recently, a high-resolution crystal structure of human CCR5 bound to the approved drug revealed a ligand-binding pocket that is distinct from the putative major binding sites for chemokines and HIV gp120, affording unprecedented insight into the mechanism of allosteric modulation of chemokine signalling and viral entry (45). This structure may suggest potential news that could further inhibit the bioactivity of CCR5 (46). In addition, a subpocket on the N-trimer of HIV-1 gp41 was identified, with implications for developing anti-HIV entry inhibitors (47). Besides targeting an unconventional binding site, another rational design strategy to combat drug resistance has been to maximize highly conserved site interactions and significantly enhance extensive H-bond interactions with main-chain atoms strategy has been extensively employed to seek a variety (48).

HIV Treatment

Current anti-HIV medicines inhibit critical phases in the HIV life cycle; nevertheless, HIV can mutate, leaving these medications ineffective (49). HIV therapy is typically administered with two or three groups of ARVs, a process known as cART (50). ARVs are classified into five types: non-nucleoside reverse transcriptase drugs, protease inhibitors, entry/fusion inhibitors, integrase inhibitors, and nucleoside/nucleotide reverse transcriptase agents (51). The three medications of choice are an integrase-strand transfer blocker and two nucleoside reverse transcriptase inhibitors (52). ARVs are administered regularly, making adherence challenging (53). Any disruption in this everyday routine may result in the virus resurfacing (54). ARVs are administered orally; hence absorption is the primary method (55). Long-acting injectables (LAIs) such as Cabenuva, on the other hand, are injected intramuscularly rather than orally, giving LAIs an advantage over orally administered

Table 1. Substances retrieved from PubChem have been shown to inhibit HIV-1 protease in vitro.

Compound	Compound ID	Molecular Formula	Structure	HIV Protease activity
arylsulfonamide 15	CID480447	$C_{35}H_{49}N_3O_{11}S$		Active
arylsulfonamide 16c	CID514961	$C_{32}H_{45}N_3O_{11}S_2$		Active
arylsulfonamide 11b	CID480469	$C_{32}H_{46}N_4O_9S$		Active
arylsulfonamide 16b	CID480440	$C_{33}H_{46}N_4O_{10}S$		Active
arylsulfonamide 13	CID480441	$C_{32}H_{43}N_3O_{11}S$		Active
CHEMBL60433	CID478338	$C_{21}H_{22}N_2O_6$		Inactive

medicines (56). The ARVs' biodistribution was also studied (57). New research, the first to evaluate ARV concentration from human brain tissues, found a greater concentration than any previously reported concentration (58). Furthermore, various ARVs might be more concentrated in different tissues, implying that particular phases in the HIV life cycle are not inhibited in specific reservoirs (59). As a result, ARV treatment considers medication-to-drug interactions, which may increase drug toxicity (60). Furthermore, some HIV patients use marijuana medically or recreationally, which can block the cytochrome P450 enzymes (61). This can eventually lead to increased ARV concentration in the circulation, which increases adverse effects and excretion rates (62).

Therapy prediction engines

A virtual phenotype is an estimate of the result of a laboratory experiment that serves as the foundation for selecting a suitable therapy in a second manual phase (63). The goal of therapy prediction engines is to automate the second stage (64). They rate various therapeutic alternatives in terms of their chance of success for a particular patient (65). Therapy prediction engines tackle a considerably more complex problem than virtual phenotypes since they try to predict clinical outcomes rather than merely a laboratory readout (66). The caretaker then chooses an appropriate therapy from the top-ranking treatments supplied by the prediction engine (67). In doing so, she will consider patient criteria the prediction engine does not evaluate, such as adverse reactions and ease of use (68).

The early treatment prediction engines constructed resistance ratings from virtual phenotypes relatively simply (69). Examples include the genotypic susceptibility score (GSS), a normalized sum of the virus's resistance ratings against several treatment types (70). More advanced systems use cutting-edge statistical learning methods to provide a prediction which involves both the estimated viral resistance and more details, such as drug interactions and an estimate of the virus's expected evolutionary development to escape therapy in the future (71). Therapy forecasting systems can use the predictions provided by virtual phenotypes to predict therapy efficacy (72). Still, they can also use clinical correlates, information on patient history - such as previous use of drugs or combinations of drugs and previously observed resistance mutations - and even information on patient genotypes - such as HLA alleles (73). Several therapeutic prediction systems (THEO from the geno2pheno suite, the EuResist prognosis engine, and the RDI TREPS system) have been published and are available on the Internet (74). Furthermore, positions are under pressure from HLA presentation and certain antiviral medications (75).

CONCLUSIONS

Computer-assisted HIV treatment is at the forefront of personalized medicine. It is distinguished by complicated genomic biomarkers - essential portions of the viral genome - and a wide range of therapeutic alternatives. The therapy decision problem is dictated by viral resistance and is difficult, if possible, to solve manually. There are two versions of treatment selection systems. The first generation of virtual phenotypes predicts the virus's resistance to any given medicine in the arsenal. Virtual phenotypes are now used in clinical settings. The second generation of therapy prediction engines combines information about a patient, such as resistance estimations, patient history, and clinical correlations. Therapeutic prediction engines, which forecast the likelihood of therapeutic success, are the subject of much research. They are currently employed in research settings but have yet to reach clinical use.

The technique that has proven effective for HIV therapy can potentially be used to treat other infectious illnesses. A fast-increasing arsenal of antiviral medications is developing for HCV infection, leading to hepatitis C and hepatocellular cancer, and combination treatment therapy will become commonplace in the coming years. The geno2pheno

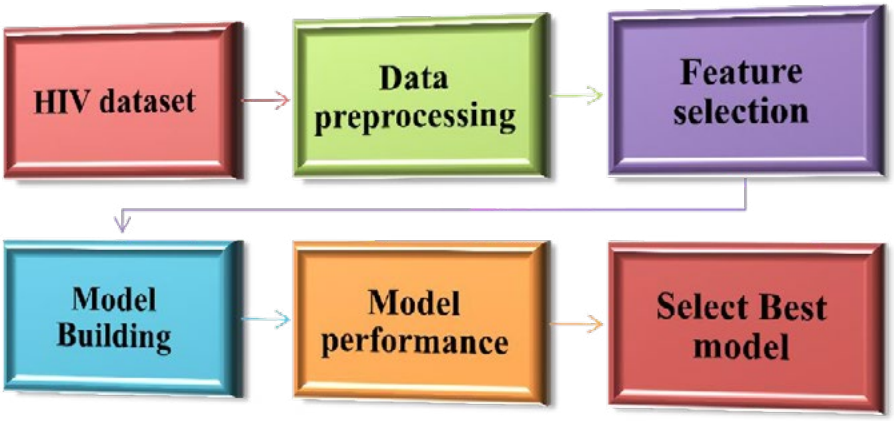


Fig 1. Flowchart of machine learning for HIV-failure prediction based on personalized medicine.

service already provides a virtual phenotype of HCV treatment resistance based on guidelines. With more phenotypic resistance data, we are prepared to give a mathematical model of drug resistance on that server. However, it is unknown if such a change is as essential for HCV as it is for HIV. There is optimism that individuals can quickly be cleansed of the virus using extremely efficient combination therapy treatments against HCV. This might reduce the requirement for computer-assisted therapy selection, as shown in TB when essential tabular criteria for medication administration suffice. Another situation in which this technique may be helpful is the HBV infection leading to hepatitis B, although its importance is unknown.

Using this kind of technology to combat tumors in the future has enormous promise. Cancer is similar to an HIV infection in that a parasite genome gains over the management of the cell, develops quickly, and escapes to resistant versions when challenged with medication therapy. The parasitic genome in cancer is that of the tumor cell. Compared to HIV, the genome and the pathways for resistance development are far more complicated and varied. Both situations share the problem caused by the variability of the parasite genome population. In this regard, the links between HIV and cancer are further examined.

Acknowledgements

The authors would like to thank the Biotechnology Research Centre staff members of the Parsian BioProducts Company (PBP) in Iran. This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data generated or analyzed during this study are included in this article.

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Summer 2023, Volume 8, Issue 30 (32- 40)

Adjustment of a Fibrosis Marker, Pro-Inflammatory Cytokines, and IgE in Asthmatic Animals

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DOI: 10.22034/pmj.2023.2009908.1010

Submitted: 2023-08-23
Accepted: 2023-09-19

Keywords:
Asthma
Alpha-smooth muscle actin
Mesenchymal stem cell

Abstract:
Background: A lot of patients are suffering from asthma. For decreasing the asthma symptoms, we studied the effects of conditioned medium (CM) of human amniotic membrane mesenchymal stem cells (hAM-MSCs) as a source of anti-inflammatory cytokines on splenocyte and lung tissue of asthmatic Balb/c mice.
Methods: Forty mice were categorized into four groups; ovalbumin (OVA)-induced asthma, CM-treated asthma, DMEM (Dulbecco's Modified Eagle Medium)-treated asthma, and saline control. Each group received related treatment. The lung alpha-smooth muscle actin (α-SMA) and splenocyte inflammatory cytokines and IgE were examined through Western blot analysis.
Results: Western blot showed α-SMA overexpression in the OVA and DMEM groups compared with the saline group. CM therapy could significantly reverse it compared with OVA and OVA+DMEM categories by elevating IL-10 and IFN-γ and reducing IL-4, IgE, and TGF-β .
Conclusion: CM treatment could improve asthma symptoms by adjusting α-SMA in lung tissue and pro-inflammatory cytokines and IgE in splenocytes.

INTRODUCTION

More than 300,000 patients worldwide are suffering from asthma (1) as an uncomfortable respiratory inflammation of the airways (2) with a high mortality rate (3). Several cells including eosinophils, mast cells, B lymphocytes, activated T lymphocytes, neutrophils, airway epithelial cells, as well as airway smooth muscle (ASM) can develop remodeling and narrowing of the airway, bronchial hyper-reactivity (BHR), and fibrosis (4); and T helper (Th)1/Th2 imbalance and increased inflammatory cytokines are essential in its pathogenesis (5). Thus, asthma is characterized by infiltration of inflammatory cells and overproduction of various cytokines like Interleukin (IL)-4, IL-5, and IL-13, as well as Immunoglobulin E (IgE) (3). Among imbalanced cytokines, IL-4, as a Th2 cytokine, plays the most important role in inflammation and airway remodeling and leads to airway hyper-responsiveness (AHR), infiltration of inflammatory cells, and secretion of mucus into the lungs (6).

Inflammation of the airways causes tissue remodeling and structural changes such as the thickness, increase of the basement membrane, and elevation of collagen density (7). Proliferation of the airway smooth muscles is the most important factor related to asthma exacerbation and decreased lung physiological function. This thickening is due to hypertrophy, hyperplasia, as well as increased extracellular matrix (ECM) protein deposition. In addition, airway smooth muscle in asthmatic patients is associated with the infiltration of inflammatory

cells like mast cells, eosinophils, and lymphocytes (8). Airway smooth muscle remodeling is considered an asthma hallmark and could be detected by the deregulation of proteins such as alpha-smooth muscle actin (α-SMA) (9). α-SMA is considered a smooth muscle cell marker and demonstrates myofibroblast differentiation. As α-SMA is expressed by Myofibroblasts as a differentiated form of fibroblasts, it plays a role in fibrogenesis (10), and its high expression indicates more differentiation of fibroblasts to myofibroblasts (11).

On the other hand, the spleen plays an important role in allergic response induction and pathogenesis of allergic conditions. Studies show that in inflammatory conditions such as allergies, spleen-derived mast cells produce IL-4, IL-6, and Tumor Necrosis Factor-alpha (TNF-α) in response to receptor-bound IgE. In allergic inflammation, a decrease in Interferon-gamma (IFN-γ) intensifies the Th2 response (12). In addition, IFN-γ is classified as a prototype of Th1 cytokine (5). Fluctuation of these cytokines in the spleen is an indicator of immune system activities (13).

For many years, high-dose corticosteroids, along with beta-agonists, were used to treat asthma, but they failed. So, some novel therapies exert their influence by focusing on the control of lymphocyte functionality and cytokine release; in this regard, stem cells have shown promising applications in the therapy of various human disorders (1). Among different kinds of stem cells, stem cells derived from the Human amniotic membrane (hAM) have advantages because hAM can be obtained easily after birth, and in normal situations it is not used, a small piece of this membrane has a lot of single stem cells, and hAM causes less immunogenic reactions (14).

Although stem cells have some disadvantages, for example, some shortages may occur in the tissue banks and they are not cost-effective (15),

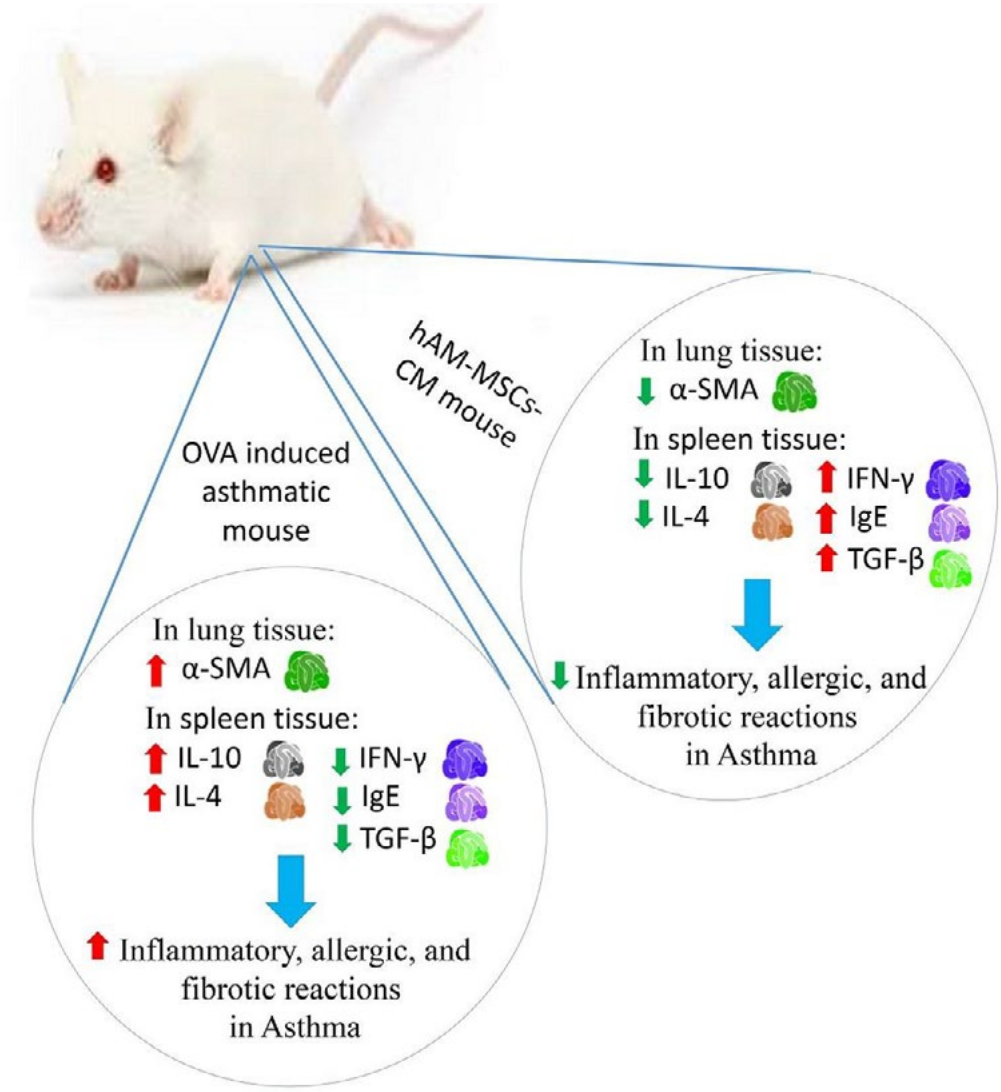

Fig 1. A graphical abstract of our study. hAMSCs-CM improved asthma symptoms by adjusting α-SMA in lung tissue and IL-10, IFN-γ, IgE, IL-4, and TGF-β in splenocytes in an OVA-induced asthmatic mouse.

the age and health status of donors and recipients affect them, they need invasive techniques, they are easily contaminated, in some cases, they show poor homing ability, low transplantation efficiency, and uncontrolled viability in vivo (16).

Researchers are exploring hAM-MSCs as a viable research and treatment option due to their high cytokine secretion and fewer ethical concerns. To address their limitations, they have introduced conditioned medium (CM), a cell culture medium that contains secretory factors from these cells, which has demonstrated therapeutic effects similar to stem cells (17, 18). CM derived from different stem cells can improve health problems like acute and chronic hind limb ischemia and myocardial infarction (18).

This study aims to overcome the current practical challenges in asthma treatment by using hAM-MSCs-CM to alleviate lung fibrosis and spleen inflammation in asthmatic mouse models for the first time.

This research is innovative in two different aspects; it is assessing the effect of hAM-MSCs-CM on asthma improvement via adjusting spleen secretion in animal models for the first time. Furthermore, as most asthma treatments are based on relieving the inflammatory and fibrotic symptoms of airways (19), it evaluates several immune responses, as well as inflammatory and fibrotic factors to assess the effectiveness of our innovative treatments.

We hypothesized with the results of this investigation, we will fill the gap of knowledge about the therapeutic effect of hAM-MSCs-CM on inflammatory factors of spleen culture supernatant and α-SMA expression in ovalbumin (OVA)-associated asthma in vivo. A summary of this study has been presented in Fig. 1.

MATERIALS AND METHODS

Preparation of the hAMSCs-CM

The hAM-MSCs and their CM were obtained and processed using established methods (17, 20). Briefly, the amniotic membrane was obtained from women with natural childbirth or elective cesarean. All donors of hAM-MSCs signed informed consent before donation. The clear vascular-free membrane was separated, washed with phosphate-buffered saline (PBS), fragmented into smaller pieces, and treated with 0.05% trypsin-EDTA. After centrifugation, the cells were washed with PBS and passed through 100µM disposable mesh. The collected cells were cultured in flasks containing high glucose DMEM, 10% FBS, Penicillin/ Streptomycin, and 10 ng/mL EGF (Royan Institute, Tehran, Iran), and then, incubated in a CO2 incubator at 37°C. After 24 h, non-adherent cells were removed, and a fresh medium was added to the culture and incubated for 48 h. Subsequently, the culture was treated with 0.25% trypsin-EDTA, and the third passage was supplemented with serum-free α-MEM medium to obtain CM. After 48 hours of incubation, the cell-free CM was obtained by filtering the supernatant through a 0.22µM filter (21).

Animal grouping and sensitization protocol

Forty male Balb/c mice (25-30 g) were purchased from Iran University of Medical Sciences (IUMS), and were categorized into four groups (10 in each group); in three groups, respiratory allergic asthma was induced by intraperitoneal injection of ovalbumin (OVA), followed by daily respiratory challenge with OVA (Sigma-Aldrich, St. Louis, MO, USA). Mice were intraperitoneally sensitized with OVA (20 µg) combined with aluminum hydroxide (2 mg) (Sigma-Aldrich) in normal saline (1ml) on days 1, 8, and 14. Then, they were daily exposed to inhalation of 3% OVA with a nebulizer (Omron CX3, Japan) in the plexiglass box from day 21 up to one week (30 minutes a day). The controls were undergone the same challenge with normal saline instead of OVA (22). Then, one of the asthma-induced groups was kept untreated (OVA) and the other groups were treated either with hAM-MSCs-CM (OVA + CM) or fresh cell culture medium (OVA + DMEM). The two treatment categories were administered two doses of either CM or DMEM intravenously (i.v), each consisting of 50 µl, on days 28 and 29.

Measurement of α-smooth muscle actin (α-SMA)

On day 30, the animals underwent surgical anesthesia. The lungs were lysed in RIPA to evaluate the α-SMA level. Approximately 20 µg of the lysates were run on SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad, CA, USA). Then, the membranes were blocked with 5% BSA (Sigma Aldrich, MO, USA) in 0.1% Tween 20 for 1 h and were incubated with rabbit polyclonal anti-α-SMA (Abcam, ab5694), as well as rabbit polyclonal anti-beta actin antibodies (Abcam, ab8227) for 1h at 25^{O}C. Then, membranes were washed three times with PBS containing 0.05% tween 20, and incubated with HRP-labeled goat anti-rabbit IgG (Abcam, ab6721) secondary antibody. The membranes were incubated with enhanced chemiluminescence (ECL) substrate for 1–2 min and evaluated by a chemo-documentation system. Protein bands were normalized to β-actin bands. Bands' densitometry was measured by the gel analyzer Version 2010a software (NIH, USA), and the percentage of the area under the curve (AUC) of each α-SMA was divided by the related β-actin band, and subsequently, calculated values were compared between groups.

Spleenocyte culture and cytokine analysis

The removed spleens were placed in RPMI and

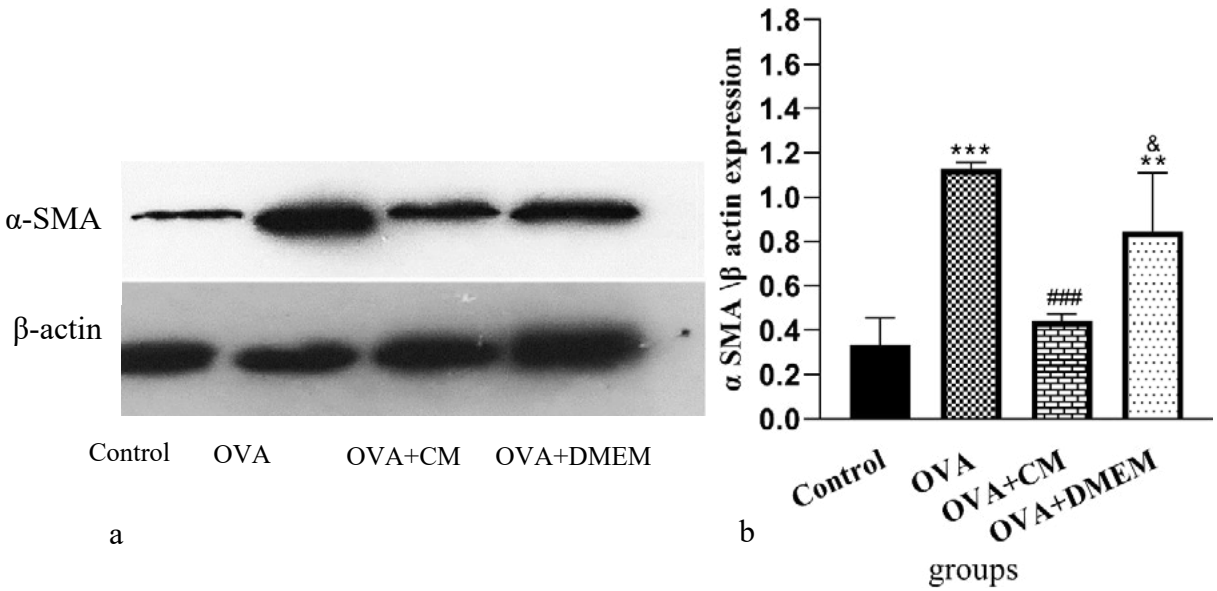

Fig 2. The effect of hAM-MSCs-CM on the α-SMA level in OVA-sensitized mice. (a) Western blots of the α-SMA. (b) Densitometric histograms of the western blot bands.
**P<0.01 and ***P<0.0001 vs. control group, ###P<0.0001 vs. OVA group and &P<0.05 vs. CM-treated one (OVA+CM). OVA: Ovalbumin; CM: conditioned medium.

the splenocytes were released by perfusion method. Splenocytes were then washed and approximately 10^6 cells were resuspended in 1 mL complete medium and cultured in a 24-well plate. The plate was incubated for 48 h in a CO2 incubator. Then, the contents of each well were mixed and the level of IL-10, IFN-γ, IL-4, and TGF-β (R&D Systems, Canada), as well as IgE (Abnova, Heidelberg, Germany) in the supernatant was analyzed by ELISA based on their instructor's manuals.

DATA ANALYSIS

Data were reported as mean ± SEM. P values less than 0.5 were defined as statistically significant. Statistical analysis of data was done by one-way ANOVA as well as Tukey post hoc tests on SPSS v.22 to determine the differences between groups.

RESULTS

The effect of hAM-MSCs-CM on α-SMA expression in lung tissue

Based on our western blot results, α-SMA was markedly increased in OVA and OVA+DMEM groups compared to controls (P<0.0001 and P=0.005, respectively). CM therapy could significantly underexpress α-SMA in the OVA+CM group compared to OVA and OVA+DMEM groups (P<0.0001 and P=0.013, respectively). However, there was no significant difference between the control and CM-treated group (P>0.999), as well as OVA and OVA+DMEM groups (P=0.176) (Figure 2).

The effect of hAM-MSCs-CM on IL-10, IFN-γ, IgE, IL-4, and TGF-β levels in splenocyte culture supernatant

In the OVA and OVA+DMEM groups, IL-10 and IFN-γ were significantly reduced compared to the control group (P<0.0001 for both groups and P=0.001, P=0.004, respectively). However,in animals treated with CM, IL-10 (P<0.0001 for both groups) and IFN-γ (P<0.0001, P=0.003, respectively) were significantly increased compared to untreated asthmatic animals and animals receiving DMEM. As expected, DMEM treatment did not significantly increase these cytokines compared to the OVA group (P>0.999) (Figures 3a and b).

Figures 3c, d, and e also show that IL-4 (P<0.0001, P=0.001, respectively), IgE (P<0.0001 for both groups), and TGF-β (P=0.001, P=0.002, respectively) in the OVA and OVA+DMEM groups increased significantly compared with the controls. The level of these cytokines after treatment with CM was significantly reduced compared with the OVA and OVA+DMEM groups (P<0.0001 for IL-4 and IgE and P<0.01 for TGF-β).

In addition, No significant difference was observed between the OVA and DMEM treatment groups (P>0.999), and the level of these factors remained high in the OVA+DMEM group.

DISCUSSION

A lot of people are suffering from asthma as a usual respiratory condition recognized by the chronic inflammatory responses of the innate and adaptive immune cells (4). Effective treatments are still needed for this disorder, as current therapies are not sufficient.

Stem cells have achieved some successful outcomes in the therapy of various human disorders but it has some disadvantages, too. To fill this practical gap in asthma treatment, we used hAM-MSCs-CM for the

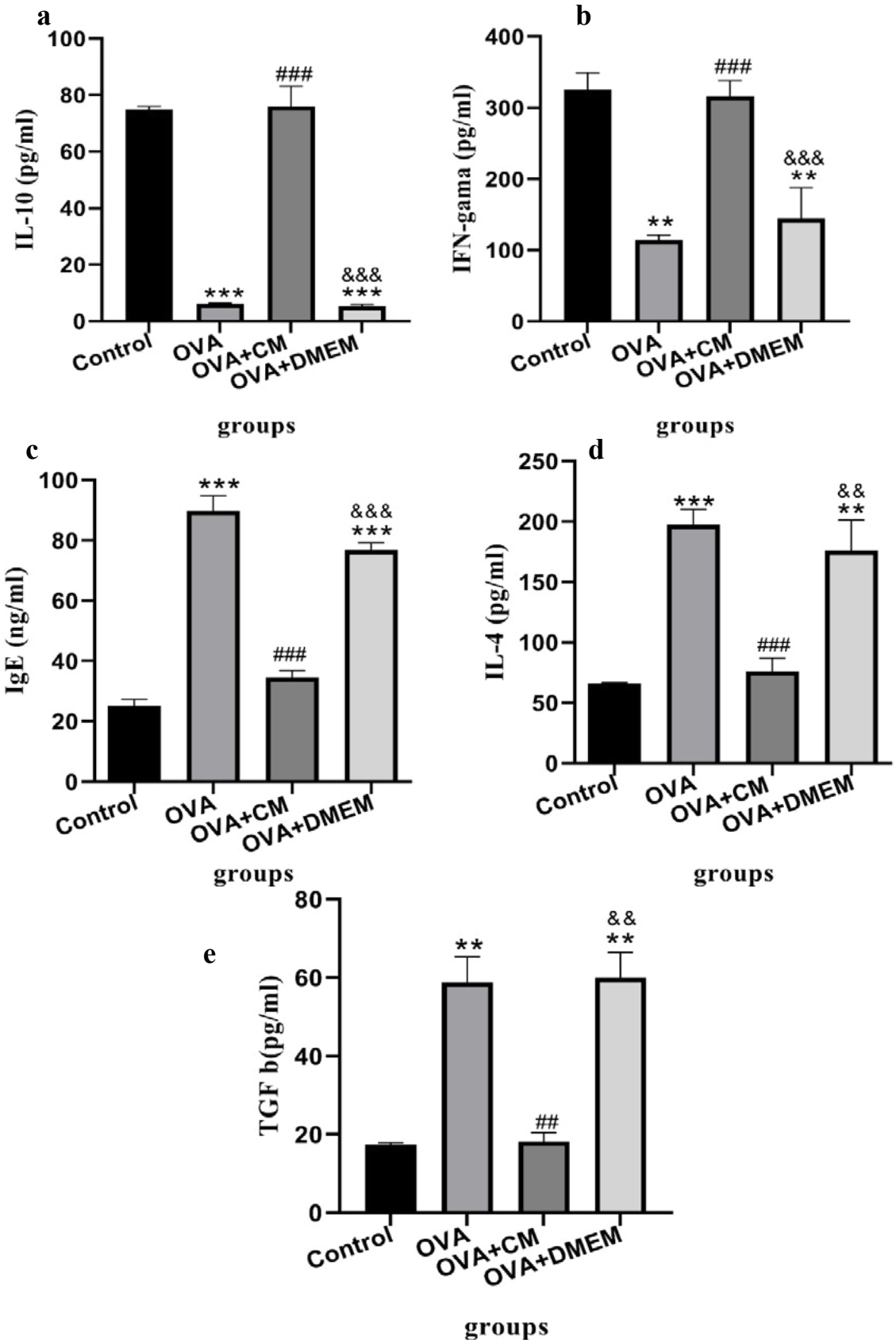

Fig 3. Effect of hAM-MSCs-CM on cytokines levels of spleen culture supernatant in the asthmatic animal model. a) IL-10, b) IFN-γ, c) IgE, d) IL-4, and e) TGF-β.
**P<0.01 and ***P<0.0001 vs. control group, ##P<0.01 and ###P<0.0001 vs. OVA group and &&P<0.01 and &&&P<0.0001 vs. group treated with CM (OVA+CM). OVA: Ovalbumin; CM: conditioned medium.

first time to decrease inflammation in the spleen and fibrosis in the lungs of asthmatic mouse models.

Bronchial wall remodeling and chronic inflammation occur at the same time with the differentiation of the fibroblasts to myofibroblasts. In recovering tissues, fibroblasts are characterized by the de novo contractile phenotype of α-SMA expression, which is associated with a lot of secretion of collagen and fibronectin as ECM components. This overactivity of myofibroblasts causes fibrosis and dysfunction (23, 24).

Our findings indicate that the use of CM improved asthma by decreasing the expression of α-SMA in the treated group compared to the untreated group. CM-treatment also increased IL-10 and IFN-γ while decreasing IgE, IL-4, and TGF-β in splenocytes. The current data confirms the data obtained in our previous study on asthmatic mice in which we analyzed these factors in the serum of animals and evaluated their lung tissue and reported that hAM-MSC-CM reduced inflammation, fibrosis, and oxidative stress (22). Our results were consistent with those of Koopmans et al., who found increased levels of IgE and Th2 cytokines, α-SMA expression, mucus production, and eosinophil infiltration in OVA-sensitized mice (8).

Studies show that α-SMA expression in asthmatic patients is higher compared with the controls. The most important finding in the differentiation of myofibroblasts from fibroblasts is α-SMA expression which its expression correlates with this differentiation. Furthermore, TGF-β plays an important role in promoting fibroblast differentiation to myofibroblasts (24). As Figure 1 shows, α-SMA was overexpressed in the OVA group compared to controls, which was significantly reduced with the CM treatment.

According to the study by Ramos-Barbón et al., there was a direct correlation between T cell proliferation and α-SMA expression in asthma airway tissue, and airway and sub-epithelial smooth muscle mass was increased in asthma (11). α-SMA can also produce TGF-β and collagen in fibrous tissues. Sun et al. showed that the amount of α-SMA in the lungs of bleomycin-induced fibrosis mice was increased compared with controls (25). Liu et al. showed that pulmonary fibrosis was associated with increased differentiation of fibroblasts to myofibroblasts, collagen secretion, and α-SMA expression. They also showed that underexpression of TGF-β could reduce the differentiation of fibroblasts to myofibroblasts and subsequently, fibrosis (26). In addition, α-SMA expression can be reduced by inactivating the TGF-β in pulmonary fibroblasts. Also, Hinz et al. reported that by TGF-β downregulation, myofibroblasts activity, and α-SMA mass are also reduced (27).

IL-10 is a pleiotropic cytokine secreted by divergent immune cells. For example, tolerogenic dendritic cells overexpress IL-10 to induce T-reg. Furthermore, for immune homeostasis, natural regulatory cell (nT-reg) secretes IL-10 in response to IL-2 (28). IL-10 can suppress allergic conditions like asthma. The administration of IL-10 with immune-regulating cells will lead to successful allergen immunotherapy (29). Based on studies, IL-10 is underexpressed in asthma pathogenesis. In severe persistent asthma, the IL-10-producing and releasing monocytes are decreased. Furthermore, IL-10 absence elevates eosinophilic inflammation of the airway and IL-5 overexpression (30).

In a study, Hou et al. overexpressed IL-10 in isolated bone marrow's mesenchymal stem cells (MSCs) and treated asthma mouse models with transgenic MSCs. They observed that eosinophilic inflammation and mucus secretion were more controlled in the transduced MSCs with a vector expressing IL-10 (MV-10) group compared to nontransduced MSCs (M) and transduced MSCs with expression vector (MV) groups. Based on this research, MSCs managed allergic asthma pathophysiology with their immune-modulatory characteristics, and IL-10 overexpression could testify to this effect (30).

Furthermore, our study found that reducing IgE in the CM group was a significant achievement . As IgE plays crucial role in allergic reactions, its reduction or neutralization can help manage the outcomes. The first approved humanized mAb which was administered to patients suffering from moderate to severe asthma was Omalizumab. It inhibited IgE function via binding to the Fc fragment of free IgE. So, IgE could not bind to the high-affinity FcεRI receptor on the surfaces of inflammatory immune cells like mast cells and basophils (31).

The spleen is involved in allergic diseases, as splenic immune responses play pivotal roles in their development. Based on studies, in inflammatory conditions such as allergies, mast cells grow in the spleen and produce IL-4, IL-6, and TNF-α in response to receptor-bound IgE. *In vitro*, stimulation of spleen cells in OVA-sensitized mice led to the development of mast cell populations in the spleen. Mast cells play a vital role in the Th2 induction, IL-4 overproduction, and Th1/Th2 imbalance. IFN-γ inhibits Th2 differentiation, so a decrease in IFN-γ intensifies the Th2 response and ultimately, causes allergic inflammation (12). Studies have shown that in OVA-sensitive mice, spleen cells secrete more IL-4, IL-5, IL-13, and IgE (32). Based on Gauvreau et al. research, IL-33 as an epithelial cell-derived mediator also can worsen asthma by inducing basophil-related IgE and IL-4 (33). The study by Li et al. showed that IL-4 was significantly upregulated in the spleen of the OVA group compared with controls (6). In the

study of Yun et al., the spleen cell culture supernatant IL-4 in the OVA group was more than in the controls, while IFN-γ was less than in the controls (5). Figure 2 shows our results in line with this content. IL-4, IgE, and TGF-β of splenic culture supernatant in the OVA group were higher than controls and IFN-γ and IL-10 were less than controls, and CM treatment reversed the results.

A decrease in IFN-γ and an increase in IL-4 can be induced by differentiating Th cells which are stimulated by Dendritic cells in the spleen as a secondary lymphoid organ and can be involved in many diseases, such as asthma, by affecting the immune response (34). Decreased number and functional defects of T regulatory (Treg) is a key cause of asthma. Evidence suggests that asthma is associated with Treg and its immune disorder (35). There is evidence for the role of Treg in maintaining immune tolerance in allergic diseases (16, 36). The study of Kianmehr et al. showed that in the presence of OVA, due to Th1/Th2 imbalance and decreased Treg function IL-4, TGF-β, and IL-17 were elevated in spleen cells of asthmatic animals and IFN-γ and Treg were decreased (37). Treg mediates the immune response by controlling the secretion of cytokines. Studies show that Treg levels in the OVA category are lower than that in controls. Jing's study also showed that Treg depletion in splenic lymphocytes can be effective in Th1/Th2 imbalance [5]. When naive CD4+ T cells express IFNγ they convert to the T helper type–1 (Th1) cells, while by expressing IL-4, they are converted to the Th2 cells (38). As in our study CM could increase IFNγ and decrease IL-4, we can conclude that the immune response was toward inducing Th1 and Asthma improvement. Cazzola et al. also introduced anti-TNF-α and Th1 cytokine-directed therapies as effective asthma treatment (39).

Previous studies have shown that MSC induces Treg in both mice and humans, both in vitro and in vivo. The elevated number of Treg and its activity are associated with MSC-regulated immunity. The study by Dai et al. shows that Treg/T lymphocytes in the spleen of asthmatic animals are significantly less than the healthy ones and MSC administration improved the percentage of Treg and reduced splenic cell infiltration [35]. According to the study by Huang et al., MSC and MSC-CM therapy share a common feature of significantly reducing macrophages in the liver and spleen. This way, MSCs are responsible for down-regulation proinflammatory macrophages, such asswitching proinflammatory macrophages (M1) to anti-inflammatory macrophages (M2) (40).

According to our results, CM can regulatethe expression of α-SMA protein and proinflammatory and antiinflammatory factors in asthmatic mice induced by OVA. Thus, our previous study and this one provide evidencethat hAM-MSCs-CM can improve asthma in mice.

Acknowledgments

This research was granted by the Iran University of Medical Sciences, Tehran, Iran. All animal studies were done under the Animal Care Committee of this university with the ethics code of no. IR.IUMS.FMD.REC.1398.050.

Ethical concerns

All animal studies were done under the Animal Care Committee of this university with the ethics code of no. IR.IUMS.FMD.REC.1398.050.

Conflicts of Interest

There is no conflict of interest.

Funding/support

This research was granted by the Iran University of Medical Sciences, Tehran, Iran (Grant code: 97-4-4-12851).

Data availability

All data is available.

Ethical statements

This investigation was approved by the Ethics Committee of the Iran University of medical science. All ethical principles are considered in this article.

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http://pmjournal.ir Original Article

Summer 2023, Volume 8, Issue 30 (41-47)

Pathogenesis of Autoimmune Thyroid Disease (AITD) in Type 1 Diabetes: The Role of Autoantibodies (anti-TPO and anti-TG) and Cytokines (IL-10, IL-6, and TNF-α)

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DOI: 10.22034/pmj.2023.2011813.1017

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Submitted: 2022-09-01
Accepted: 2022-09-23

Keywords:
Anti-TG Antibody
Anti-TPO Antibody
IL-10, TNF-α
Type 1 Diabetes Mellitus

Abstract:

Objectives: Diabetes mellitus type 1 (T1DM), which is also an autoimmune disorder, can coexist alongside other types of autoimmune diseases. This study aimed to investigate the possibility of a subclinical association between diabetes disease and autoimmune thyroid dysfunction. The clinical condition of the patient and their approach to managing their diabetes were specifically considered when deciding whether or not the patient had autoantibodies.

Methods: This study included sixty individuals who were diagnosed with diabetes type 1. (thirty males and thirty women, with a mean age of 21.04 years) and 30 healthy controls (12 males and 18 females).

Results: Diabetics had considerably greater serum IL-10, IL-6, and TNF-α levels than healthy controls. Stepwise regression indicated significant positive correlations between IL-10, IL-6, and TNF-α with these antibodies and strong inversed relationships between IL-6 and Anti-TPO, Anti-TG, antibodies.

. No matter if the antibodies were present or how severe they were, this held true. The study's findings lend credence to the idea that people with type 1 diabetes should have their thyroid antibodies and function checked.

Conclusions: Thyroid antibodies were most common among type 1 diabetics aged 21–35, according to our study (Anti-TPO and Anti-TG). IL-10, IL-6, and TNF-α levels in diabetic patients and controls were significantly different (P<0.01). IL-10, TNF-α, HbA1C, and body mass index positively correlated with thyroid antibodies, except for IL-6. Thyroid antibodies and functional abnormalities should be tested often in type 1 diabetics due to the high occurrence of thyroid autoimmune illnesses.

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune illness that has been recognized for over 30 years(1). Autoantibodies targeting beta-cell components and endogenous insulin are hallmarks of this condition, which also involves the self-destruction of pancreatic beta cells (2). Both insulin and thyroid hormones play a role in the metabolism of cells, and changes in either can perturb or impair the other's operation (3). It is estimated that between 8.2% and 9.8% of people in the general population have thyroid disorders (4). Furthermore, autoimmune conditions including autoimmune thyroiditis and autoimmune adrenal insufficiency are more common in people with type 1 diabetes, and the emergence of any of these conditions can have a significant impact on how diabetic patients are treated (5). According to information from the National Institute of gastrointestinal, diabetes, and kidney disorders, one out of every 400 to 600 kids and teenagers would be given a diabetes diagnosis in 2005. By 2025, there will be 300 million diabetic patients globally, up from the anticipated 124 million in 1997, according to the World Health Organization (6, 7).

The thyroid gland is a prominent site of damage caused by autoimmune disorders (8). The two most prevalent types of autoimmune thyroid disease (AITD), which is an immunological reaction to the thyroid brought on by an immune system dysfunction, are Graves disease (9) and Hashimoto thyroiditis (HT)(10). Some studies have suggested that thyroid dysfunction increases the severity

of diabetes in T1DM when it coexists with ATD; however, other investigations have not confirmed this association (11).

Two of the most frequent thyroid autoantibodies, thyroglobulin antibody (TG-Ab) and thyroid peroxidase antibody (TPO-Ab), have been linked to the development of allergic rhinitis, chronic spontaneous urticarial, atopic dermatitis, as well as Grave's disease and Hashimoto thyroiditis (HT)(12, 13). This study investigated how proinflammatory cytokines (IL-6, IL-10, and TNF-α) and thyroid autoimmune disease antibodies affect Type-1 DM in Iraqi patients.

METHODS

Patients and Sampling

A case-control study data collecting taking place between May 1 and October 15, 2022, a case-control study involving 60 patients with type 1 diabetes (30 males and 30 females) and 30 healthy controls (12 males and 18 females) was performed.

A vein puncture was used to obtain five millilitres of blood, which was divided into two tubes for the HbA1C test and the gel tube centrifugation. The sera were then kept at -80 degrees Celsius for biochemical and immunological analysis.

Exclusion Criteria

Patients with other autoimmune diseases, severe systemic illnesses, malignancies, and other autoimmune problems such as psoriasis, Behçet's disease, and bullous diseases that significantly reduce the quality of life were eliminated. Patients who had received immunological preparations during the preceding three months and were pregnant or were nursing were also excluded.

Ethics

Each patient signed an informed consent form before the trial. Age, sex, medical and pharmaceutical histories, and other demographic information about the patients were recorded.

Measurement of target factors

Measurement of Blood glucose

The enzymatic colorimetric method, which was used to assess the glucose level in the venous blood, made use of Randox diagnostic tools (14). In the presence of glucose oxidase, glucose is measured after it has undergone an enzymatic oxidation reaction. A red-violet quinone imine dye is created when the peroxidase-catalyzed hydrogen peroxide produced is combined with the phenol and 4-aminophenazone. The test tube was then filled with ten microliters of serum and 1000µl of the working solution. After being incubated for ten minutes at 37oc, the absorbance was measured at 500 nm.

Measurement of Hemoglobin A1c test (HbA1C)

The Cobbas C411 in vitro diagnostic test device quantified total and mmol/mol hemoglobin A1c by a photometric transmission measurement. Diluted blood was mixed with TRIS buffer to extract hemoglobin from erythrocytes. Materials were mixed with sodium lauryl sulfate in a reaction chamber (SLS). SLS created SLShemoglobin. SLS hemoglobin complex measurements at 525 nm were used to determine total hemoglobin concentration. HbA1C from another sample was denatured using potassium ferricyanide and sucrose laurate. The latex particle's HbA1C antibody binds to denatured HbA1C. Synthetic antigen and HbA1C antibody react to suppress latex agglutination. HbA1C concentration was measured by latex agglutination inhibition response at 625 nm. The HbA1C percentage was calculated by dividing the concentration by the total amount.

Measurement of IL-10, IL-6 and TNF-α by ELISA

40µl of sample dilution buffer and 10µl of the sample (dilution factor is 5) were added to the sample wells. Samples were loaded onto the microplate's bottom without touching the side walls. The membrane of the closure plate was sealed, well mixed, and lightly shaken followed by a 30-minute incubation period at 37ºc.

The extremely concentrated washing buffer was diluted with distilled water to get the desired consistency (30 times for 96T and 20 times for 48T). The plate was completed after the closure of the plate membrane was carefully peeled off, the membrane was aspirated, and the plate was then refilled with wash solution. The washing solution was diluted after it had been allowed to sit for a thirty-second interval. The washing procedure was repeated five times before adding 50µl of HRP-conjugate reagent to each well (except for the well that served as a blank control). This was followed by incubation as described in Step 3 and washing as described in Step 5. In the course of the coloring procedure, each well was supplied with an additional 50µl of chromogen solution A and another 50µl of chromogen solution B. After gently shaking the wells, the solutions were incubated for 15 minutes at 37°c. Avoiding light while coloring solutions. Each well-received 50µl of stop solution to terminate the reaction. The process ended. The well water suddenly changed from blue to yellow. After 15 minutes, the experiment was terminated by the stop solution, and the absorbance density was determined at 450nm.

Measurement of Anti-TPO and TG-Antibodies by ELISA

After adding biotin in the same volume, all wells received 50µl of the patient's diluted serum and the

standards. After 30 minutes of incubation at 20–32ºc, the wells were washed three times with 300 millilitres of washing buffer (diluted 1:50). The plates were incubated at 20-32ºc for 30 minutes and 100µl of the conjugate added to each well before being washed: Then, 100µl of TMB was added to each well, and they were all incubated for 30 minutes at 20-90ºc. The final step was adding 100µl of stop solution to each well and measuring its absorbance at 450 nm.

STATISTICAL ANALYSIS

An analysis using a t-test was carried out on the data (15). To investigate the connections between the variables, non-parametric Spearman correlation coefficients were utilized. We decided to use a significance level of $P<0.05$ as our cutoff for statistical significance. The mean and standard deviation of the numbers are displayed here.

RESULTS

Characteristics of T1DM patients on a general and laboratory level

Table 1 presents the characteristics of patients with type 1 diabetes. According to the data, there were 30 patients with type 1 diabetes mellitus for each gender. Even though men's average age was 10.83 and women's average age was 9.82, the average age of the entire patient group was 10.35 and 10.34 years, respectively. Table 1 also reveals that all of the patients had an average body mass index (BMI) of 21.0 kg/m2, with males having a BMI of 20.49 kg/m2, women having a BMI of 21.55 kg/m2, and kids having a BMI of 2.89 kg/m2.

The measure of HbA1C, BMI and random blood sugar in T1DM patients

In Table 2, type 1 diabetes patients with anti-TPO antibodies differ significantly from the control group in HbA1C, BMI, and random blood sugar levels. Different HbA1c and irrational blood sugar levels were seen among patients with type 1 diabetes who had anti-TPO antibodies (see Table 2).

Anti-TPO antibody prevalence in T1DM patients

Based on the data in Table 3, only 2 (3.3%) of persons with type 1 diabetes had anti-TPO positive antibodies, while 58 (96.6%) had anti-TPO negative antibodies. The absence of the antibody in the control group was statistically distinct from the presence of the antibody in the patients group ($P<0.001$).

Assessment of IL-10 in control and T1DM patients

The control group had higher serum IL-10 levels than the T1DM patients ($P<0.0001$). Although both groups had IL-10 levels in the normal range (4.8-9.8 pg/Ml), the T1DM group had considerably higher levels (Mean SD: 5.082.81±1.9050.77)(Figure 1A).

Assessment of IL-6 in control and T1DM patients

The control and T1DM patient groups had

Table 1. Characteristics of type 1 diabetics participated in the studyy

variable	Age years (mean ± SD)	BMI (kg.m^{-2})
Female (30)	9.82±12.62	21.55±2.72
Male (30)	10.83±7.81	20.49±2.89
Total (60)	10.35±10.34	21.0±2.84

Table 2. Comparison of diabetic individuals› HbA1C, BMI, and blood glucose levels based on whether or not they had positive or negative levels of anti-TPO antibody.

Parameter	Patients with positive anti-TPO Ab	Patients with negative anti-TPO Ab	Control group
HbA$_1$C (%) (mean±SD)	8.4±0.7	1.44±6.43	5.02±0.09
BMI(kg/m2) (mean±SD)	18.55±0.77	21.09±2.82	22.67±2.80
Random blood sugar (g/dl) (mean±SD)	319±57.98	324.9±90.72	100±0.0

Table 3. Percentage and number of diabetes patients having a positive and negative level of Anti-TPO Antibody.

Case	Anti TPO Ab positive Number (%)	Anti TPO Ab negative Number (%)	Statistical significance (*P*-value)
Type1 diabetic patient (n=60)	2 (3.3%)	58 (96.6%)	< 0.001
Healthy Control (n=25)	0 (0%)	30 (100%)	

significantly different serum IL-6 concentrations (P<0.0001). Compared to the healthy control group, the T1DM group exhibited considerably higher serum IL-6 levels (Figure 1B).

Assessment of TNF-α in control and T1DM patients

Statistical analysis showed a highly significant difference in TNF-α levels (P<0.0001) between the control group and the group of patients with type 1 diabetes. In contrast to the healthy individuals who served as the control group, those with type 1 diabetes had serum TNF-α much higher levels (Figure 1C).

Assessment of Anti-TPO Antibody in control and T1DM patients

Figure 2A demonstrates a substantial difference in serum anti-TPO antibody concentrations (P<0.0001) between the Control group and the T1DM patient group. Patients with type 1 diabetes had significantly more serum anti-TPO antibodies than the healthy control group.

Assessment of Anti-TG Antibody in control and T1DM patients

The levels of anti-TG antibodies in the serum of the control group and T1DM group were noticeably different (P<0.0001) from one another. Anti-TG antibodies were found in higher concentrations in type 1 diabetic patients than in healthy controls (Figure 2B).

Figure 2. A. Anti-TPO antibody levels in the sera of type 1 diabetic patients compared to healthy controls. B. Anti-TG antibody concentrations in the sera of type 1 diabetic patients compared with those of healthy controls.

Correlation of anti-TPO Ab with IL-10, IL-6 and TNF-α

According to the obtained results, there was a positive correlation between anti-TPO Ab and (IL-6, IL-10 and TNF-α) in T1DM patients (Figures 3A, B and C).

Correlation of anti-TPO Ab with IL-10, IL-6 and TNF-α

According to the obtained results, there was a positive correlation between anti-TPO Ab and (IL-6, IL-10 and TNF-α) in T1DM patients.

DISCUSSION

It is estimated that the number of people diagnosed with type 1 diabetes mellitus has increased by between

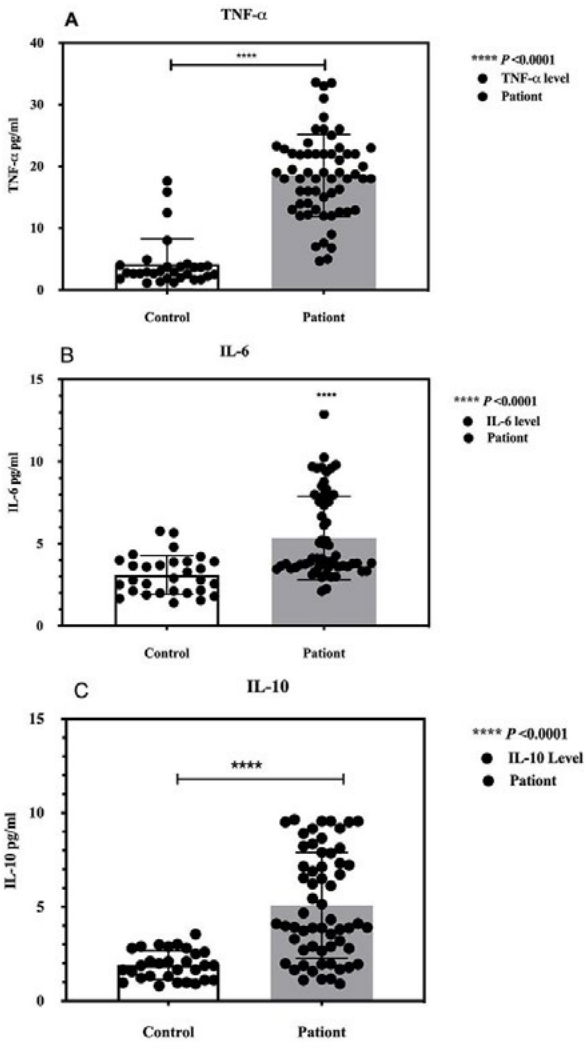

Fig1. A. Serum IL-6 levels in type 1 diabetics versus healthy controls. B. Serum IL-10 levels in type 1 diabetics and healthy controls. C. Serum TNF-a levels in type 1 diabetic patients and healthy controls.

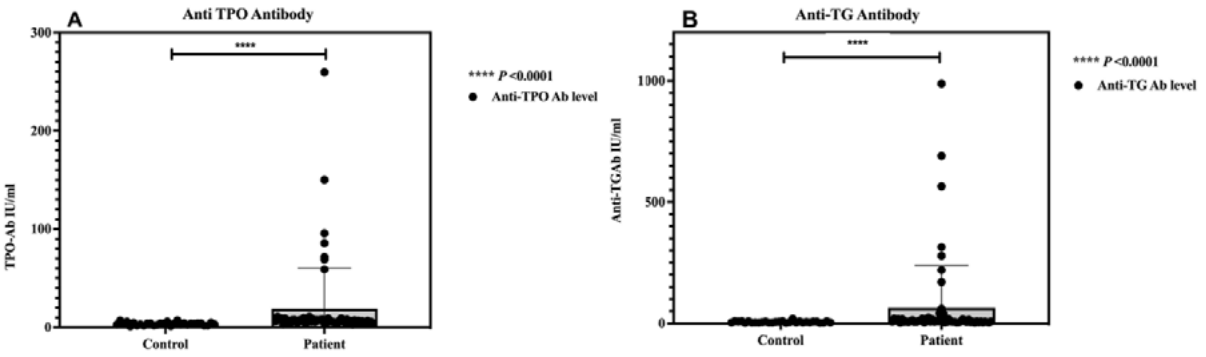

Fig 2. A. Anti-TPO antibody levels in the sera of type 1 diabetic patients compared to healthy controls. B. Anti-TG antibody concentrations in the sera of type 1 diabetic patients compared with those of healthy controls.

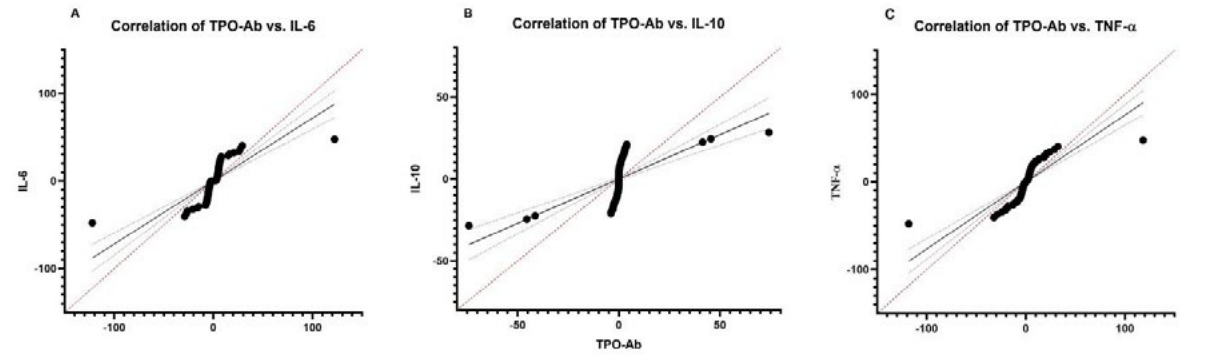

Fig3. A. Scatter plot depicting the participants› levels of anti-TPO Ab and IL-6 illustrates the link between the two. There is a close relationship between anti-TPO Ab and IL-6 (r = 1.00, P< 0.0001, positive association). B. Scatter plot showing the association between the levels of TG-Ab and IL-10 in the subjects. There is a positive association between anti-TPO Ab and IL-10 (r = 1.00, P< 0.0001, significant positive correlation). C. Scatter Plot showing the Correlation between anti-TPO Ab and TNF-α among the Subjects. anti-TPO Ab positively correlates with TNF-α (P<0.0001, r=1.00). (TPO-Ab: IU/mL, IL-6: pg/mLand TNF-α: pg/mL).

3% and 5% every year over the previous few decades. Predictions indicate that this pattern will be maintained (16). T1DM is assumed to be predominantly brought on by the autoimmune death of beta cells in the vast majority of individuals (17).

Changes in thyroid hormone levels are another potential complication of diabetes(18). Diabetic individuals who also suffer from thyroid issues sometimes struggle to maintain stable blood sugar levels (19). Both hypothyroidism and hyperthyroidism have negative effects on glucose tolerance and glucose management in diabetic individuals, however, hypothyroidism can reduce insulin requirements (20). Due to decreased gluconeogenesis and liver glucose excretion, hidden hypothyroidism causes recurrent hypoglycemia crises. Hyperglycemia occurs in hyperthyroidism due to increased glucose absorption and glycogenolysis (21).

There is a higher risk for autoimmune hypothyroidism and hyperthyroidism, and 17% to 30% of people with type 1 diabetes also have AITD (22).anti-TPO and anti-TG antibody levels are considered to be indicators of autoimmune thyroiditis(23). Evaluating these antibodies in distinct populations of adult patients with type 1 diabetes was the aim of our research(24).

Sixty type 1 diabetics without thyroid issues were enrolled in this trial. The study included 30 men and 30 women. Blood samples were collected from all subjects to check for the presence of pro and anti-inflammatory markers including TPO-antibody, TG-antibody, IL-10, IL-6, and TNF-α. Children and adolescents with type 1 diabetes had higher levels of thyroid antibodies compared to the healthy controls. Antibody positivity and autoimmune thyroiditis were investigated in this study of type 1 diabetic children and adolescents in Iraq.

TPO Ab and Tg Ab were shown to have the highest incidence among individuals with type 1 diabetes in a study that analyzed the prevalence of various autoantibodies in a sample of 814 people with type 1 diabetes.

Although no statistically significant difference was detected, the current investigation revealed that, with a ratio of 5/1, females were more likely than males to have positive serum anti-TPO antibodies. Similarly, even though there were more female patients with these antibodies than male patients, Hansen et al (25), Prazny et al (15), and Sharifi et al. (26) reported that

there was no statistically significant difference in the prevalence of positive serum anti-TPO antibodies between the sexes. In our investigation 5 (8.3%) of 60 antibody-positive patients showed antibodies against both TG and TPO. Moreover, one in sixty patients had anti-TPO antibodies and, one person had only anti-TG antibodies.

There is a lack of consensus among the published studies concerning the part that IL-10 plays in the beginning stages of type 1 diabetes. It has been demonstrated that prolonged exposure to IL-10 during an early stage in the evolution of a disease can speed up the development of that disease (27), but IL-10 exposure during the later stages of prediabetes can stop disease progression (28). Our results showed that serum IL-10 levels were considerably greater in diabetes patients than in controls. The production of IL-10 in T1DM patients may be a response to the rise in pro-inflammatory cytokines, according to He et al. (29) and Reis et al. (30) studies, which both support these findings.

Diabetes patients' blood levels of IL-6 are either higher or equivalent to those of normal people when compared to those levels (31). As a multifunctional cytokine, IL-6 is secreted by a wide variety of cell types such as T cells, macrophages and endothelial cells (32). Several studies found that low amounts of IL-6 increase insulin synthesis, while large levels block it (33, 34). Type 1 diabetics have higher serum IL-6 levels than controls in a comprehensive study. IL-6 can also damage pancreatic b cells over time by encouraging B lymphocyte growth and killer T cell activity (33). Changing blood IL-6 levels have not been linked to type 1 diabetes development. IL-6 may be a biomarker for type 1 diabetes, according to recent studies(35).

When IL-6 levels were compared between young people with type 1 diabetes and healthy controls, it was revealed that they were similar in both groups (36). However, another study involving young volunteers found that those with type 1 diabetes had much greater levels of IL-6 (37). Additionally, Bradshaw and colleagues (38) discovered that Type 1 Diabetes patients had considerably higher amounts of IL-6 release in their isolated monocytes from blood cells than did individuals in a healthy state.

Type 1 diabetes mellitus, whether it's complicated or not, is profoundly impacted by TNF-α. TNF-α levels were discovered to be lower in the blood of children with newly diagnosed type 1 diabetes mellitus compared to those without the disease; nonetheless, issues remain about its function and the validity of the presented data (39). The researchers found TNF-α serum levels comparable to healthy children. Newly diagnosed type 1 diabetic children have higher TNF-α and IL-6 levels than those with severe diabetes for a longer time(40). Our results demonstrated that type 1 diabetic patients' TNF-α levels were significantly greater than those of healthy controls and that these patients also developed antibodies against TPO and TG ($r = 1$, $P<0.0001$).

CONCLUSIONS

Patients with type 1 diabetes may be screened for autoantibodies to identify subclinical cases of AITD, however, the test's ability to predict the emergence of clinical symptoms is limited. Patients who have been found to have positive autoantibodies need to be monitored closely since there is a possibility that their diabetes control could worsen further and that their organs will become dysfunctional.

FUNDING

No funding was provided for this investigation.

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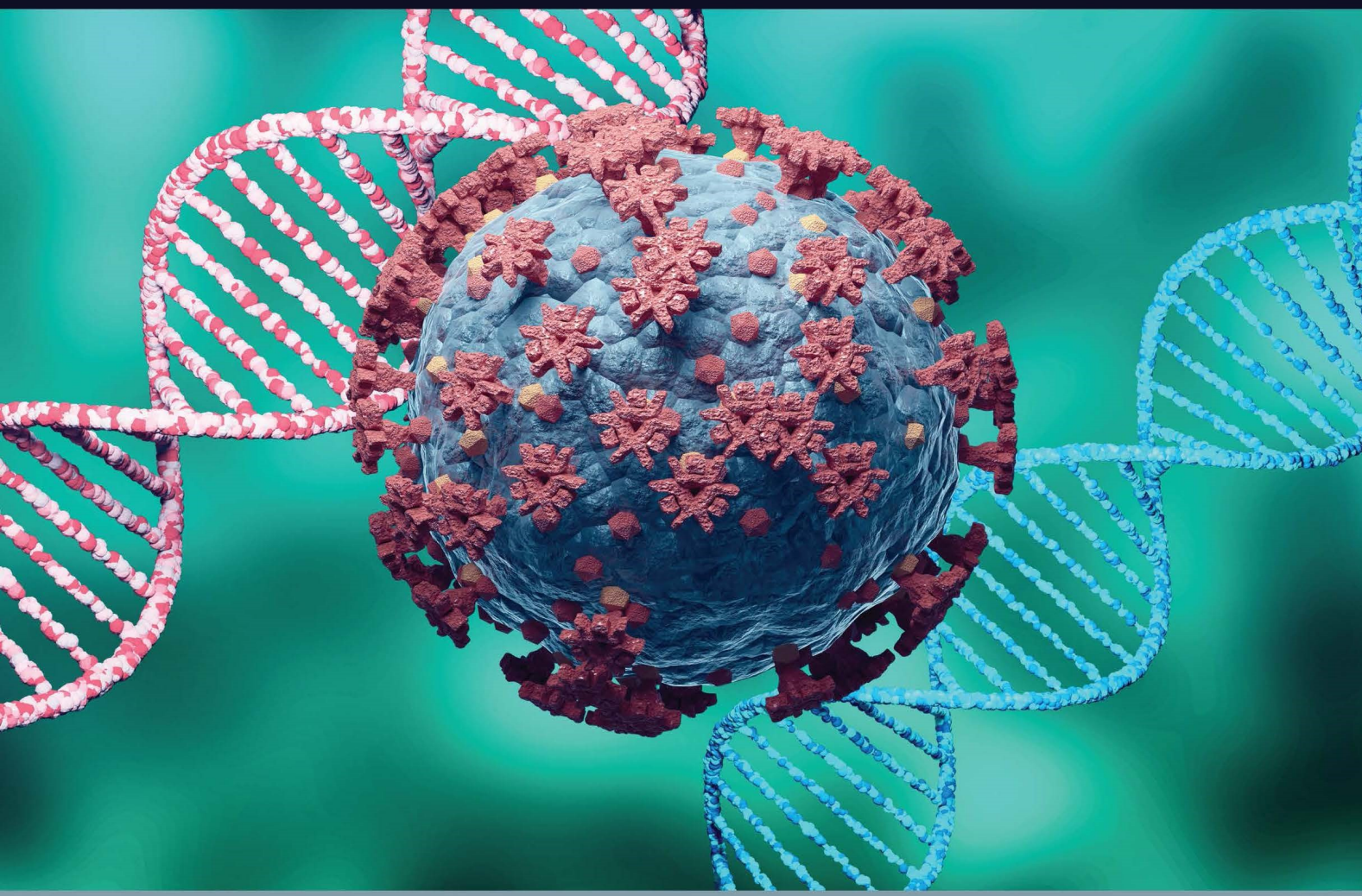

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