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Role of Biomedical Engineering During COVID-19 Pandemic

Review Article

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Abstract

In December 2019 the novel virus of corona-viruses family caused by SARS-COV-2 virus appeared in Wuhan, Hubei province, spreading rapidly throughout China, just a few weeks later it starts spreading all around the world bringing severe consequences that should be laid out and studied in order to raise the level of readiness to face the upcoming pandemics. This review paper aims to scheme out the social and the economic effects of Covid19 Pandemic, and the importance of the healthcare infrastructure in the response to the rapid spread of pandemics.In this paper the roles of 3D printing, artificial intelligence integrated in different technologies in the urgent response to pandemic like Covid-19 is reviewed. In addition, the very wide research area of vaccines and treatments are covered, reviewing the most promising candidate drugs and vaccines that entered the clinical trials to date. In coorperation with several other disciplines, biomedical engineering with its wide range of application areas played an important role in the propagation of the urgent response.
Keywords: Covid-19, SARS-COV-2, Economics Impacts, Social Impacts, Biomedical Engineering, Healthcare Infrastructure, 3D printing, Artificial Intelligence, Vaccines.

1. INTRODUCTION

Since the beginning of suspicions about the spread of a virus in China in late 2019, and its possibility of transmission between humans, people all over the world have started asking many questions about what is the nature of this virus? Is there a cure? What are the methods of prevention? How is life in quarantine? and many other questions. Unfortunately, at the moment of writing this article, deaths of this virus have reached (340 thousand) and (5.5M) infections around the world [1]. And as if we are watching a fantasy movie, the cities that have always been crowded and full of tourists, vendors and photographers, now sit empty and surrounded by a shadow of fear and anticipation as people remain indoors and avoid crowds during the coronavirus pandemic.

Our goal in this report, despite the few - and rapidly changing - sources till this day, is to give an overview, but at the same time, a comprehensive overview of all the effects of Coronavirus, with a focus on the field of healthcare and medical devices. A historical background of the virus, its ways of spreading, and its social and economic impacts on societies will be introduced. Then we will talk about the health system infrastructure, medical devices and tools used to counter the spread of the virus. Finally, we will mention the methods that have been tested and accepted in its possibility of treating the virus.

COVID-19

In late December 2019, the WHO office in China received a report of a disease that can spread between humans, which was observed in early December in Wuhan, the capital of Central China's Hubei province. Because of the strange symptoms and the lack of knowledge of its causes, the doctors called it "pneumonia of unknown etiology." And by mid-January, the spread of this disease was proven in all provinces of China in addition to many other countries [2]. On Wednesday, March 11, 2020, WHO Director-General Tedros Adhanum Gebresus announced that the United Nations organization considered the new Coronavirus, which is spreading across the globe as a " Global Pandemic". Since that time, the number of cases of infection and morality increased rapidly all around the world, Figure 1 shows these numbers.

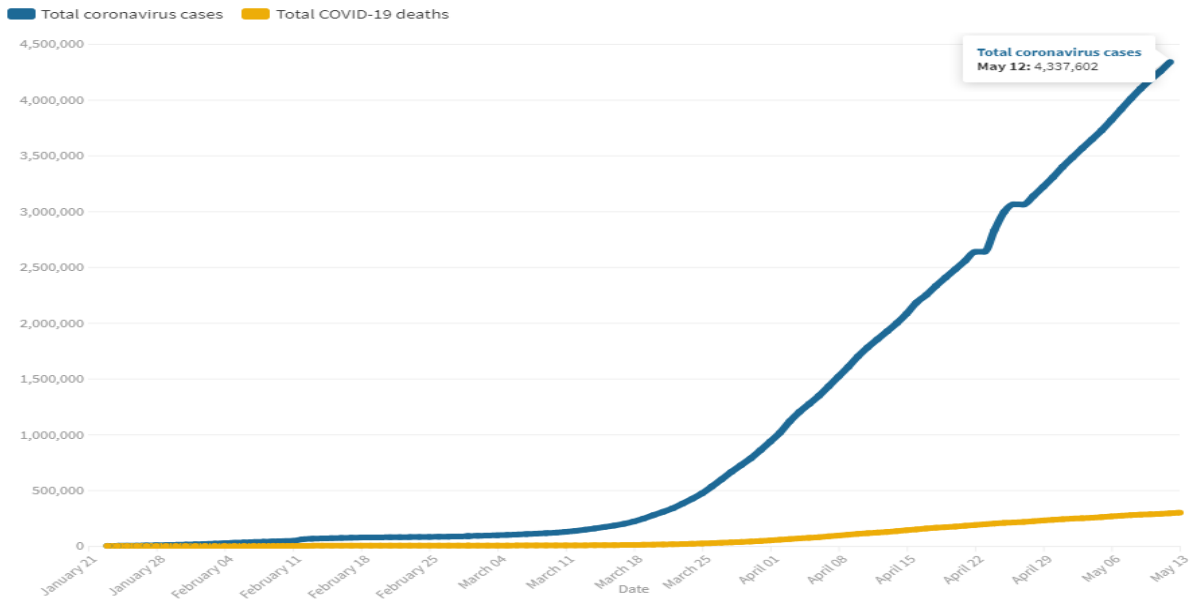

Figure 1. COVID-19 infection and deaths over time. *Euronews statistics.

Coronaviruses are a family of viruses discovered in the 1931s that infect mammals and birds. "The term coronavirus (Latin: corona, crown) was adopted for these agents, because of their characteristic fringed appearance under the electron microscope after negative staining" [3]. These types of viruses commonly cause mild (like common cold) to moderate upper respiratory tract illnesses. There are hundreds of types of coronaviruses that infect animals, such as cats, pigs, or bats. For many reasons and in different ways, these viruses can be transmitted to humans causing similar symptoms on the respiratory system. In the 1960s, first human coronaviruses were discovered [3]. According to the researchers' findings, the coronaviruses that can infect humans are:

- (229E, NL63, OC43 and HKU1); cause symptoms of the common cold.
- (MERS-CoV, SARS-CoV and SARS-CoV-2 or COVID-19); cause serious, even fatal, diseases.

Furthermore, it was confirmed that SARS-CoV and MERS-CoV originated in bats, and since they are from the same family which leads to the possibility that SARS-CoV-2 also originated in bats [4].

2. SOCIAL AND ECONOMIC IMPACTS

Epidemics used to kill millions of people, including cholera, plague and measles [5]. Despite the advancement of medical technologies and healthcare systems, the 21st century witnessed an outbreak of many infectious diseases that sparked panic around the world, including the Coronavirus, which is the last link in the series of epidemics that struck the world, killing millions of people. When the disease spreads between people and turns into a local epidemic then a pandemic, their effects are not only limited to human physical and psychological health, but also cause many crises, including economic, social, and lack of resources.

Social Impacts

Coronavirus's spread affected all groups of society, but there are groups who are more susceptible to this and other diseases than others. UN reports [6] indicate that: people living in poverty situations, older persons, persons with disabilities, youth, and indigenous peoples are the most vulnerable group that is affected by the social and economic impacts of the virus. The statistics indicate that a large percentage of deaths are from this category.

With the importance of the government's precautions to prevent the spreading of the virus, such as social distancing and the lockdowns, many news and resources point to a rise in domestic violence. After 4 months of the outbreak, The Secretary General António Guterres from the United Nations called on Twitter for urgent action to reduce domestic violence towards women; “I urge all governments to put women’s safety first as they respond to the pandemic” [7]. It is true that people may be experiencing difficult times these days because of the news or fragile resources, and that any word may be a reason for a quarrel. But this remains in the normal state among families if it does not turn into violence. The Guardian newspaper, which published an article a while ago showing an increasing in the number of domestic violence in many countries. In Russia, reported cases of domestic violence have more than doubled during the country’s coronavirus lockdown which started in the late of march. According to recent reports; “The UK’s largest domestic abuse charity, Refuge, has reported a 700% increase in calls to its helpline in a single day, while a separate helpline for perpetrators of domestic abuse seeking help to change their behavior received 25% more calls after the start of the Covid-19 lockdown” [7].

Economic Impacts

Despite the importance of many precautions made by governments to reduce the spreading of disease, this has had a major impact on the country's economy and the living conditions of the population. Any decline in the economy, will only lead to a cessation of the circulation of money, which will also lead to a decrease in the money available for use in treating people and limiting the spread of the virus. If the spread of virus continues, there would be a contraction in the economy of developed countries by 5 percent. And the production of developing countries will be reduced at least by 0.7 percent. In addition, the pandemic will likely cause an estimated ~34 million people to fall below the extreme poverty line in 2020, with more than half of this increase occurring in African countries. Also, Lockdowns, travel restrictions and the closing of national borders implemented by governments have caused a freezing in the economic activities across the board, wherefore “world trade is forecast to contract by nearly 15 per cent in 2020 amid sharply reduced global demand and disruptions in global supply chains” [6].

Therefore, it can be seen that the effects of this pandemic will not only hit the economic of poor or “developing” countries, the top economics in the world will be affected, for example, in China, where the outbreak of the virus first started, a drop by 13.5% of industrial production was shown, while the seasonally

adjusted retail sales fell by 21%. Also, different sectors have almost completely collapsed, for example, Car sales fell 92%, and restaurant sales dropped by ~95% [8].

Tourism During the Pandemic

One of the most severely affected sectors by the spread of the virus is the tourism sector, as the first step for most countries in limiting the spread of the virus was by closing borders and airports, in addition to closing all places and places that may cause gatherings between people. this of course has affected the workers, companies and projects in the tourism sector, and because of that, a loss of 100.8 million of related jobs is expected worldwide by 2020 (see Figure 2). Asia Pacific region is supposed to see the biggest loss from COVID-19, where a loss by 63.4 million of jobs is predicted, while Europe is the second hardest hit [9].

Assumptions about average daily expenditure and average length of stay of tourists were based on the Department of Tourism reports state that if the COVID-19 Pandemic lasts up to five months, the tourism industry of the Philippines will have an estimated earnings loss of about 170.5P billion (apx. 3 billion US dollar) [10]. Reem Al-Aseel, one like many small business owners, in Oakland, a city on the eastside of California, who was forced like other bakery and restaurant owners who were prevented from taking anyone inside, and they had to work for only limited hours, and reduce the number of their employers. As Assil puts it, “The money is running out, and there’s no sight of the money coming in.” [11]

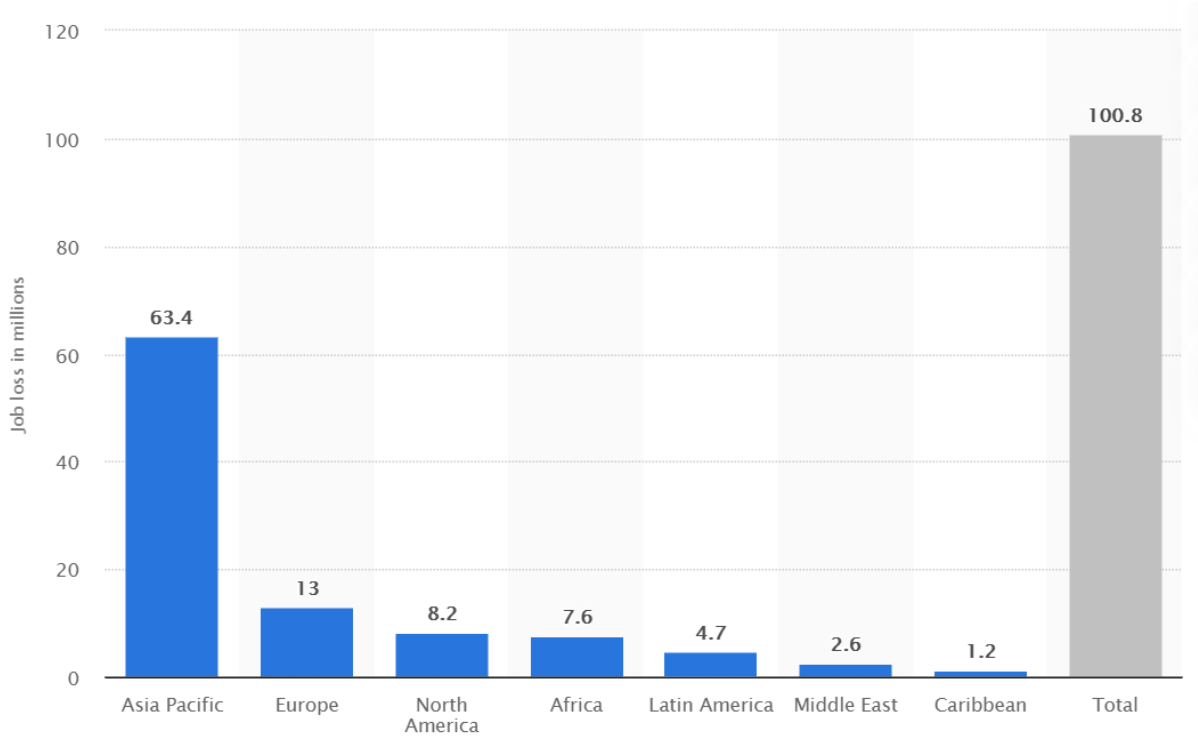

Figure 2. Predicted employment loss in the travel and tourism industry due to the coronavirus (COVID-19) pandemic worldwide in 2020, by region (in millions) [9].

Healthcare Infrastructure

The methods of dealing with the Coronavirus crisis varied from countries to countries on a large scale. Some governments have resorted to restricting all unnecessary internal moves. And not only is the move from one city to another, but the movement has stopped within the major cities around the world with the application of restrictions on the movements of citizens and communication within society. A curfew has been imposed for certain days or stings of the day, with the suspension of schools and some places that

may cause the transmission of the virus, such as restaurants, cafes and cinemas. Table 1 summarizes the most used different public health measures [12].

Table 1: Non-Pharmaceutical public health interventions to control disease outbreaks, adapted from Cetron and Simone [12]

Isolation	Separation of ill persons with contagious diseases from non-infected persons	• To interrupt transmission to non-infected persons . • Effective for infectious diseases with high person-to-person transmission where peak transmission occurs when patients have symptoms.
Quarantine	Restriction of persons who are presumed to have been exposed to a contagious disease but are not ill, either because they did not become infected or because they are still in the incubation period	• To reduce potential transmission from exposed persons before symptoms occur . • It is most successful in settings where detection of cases is prompt, contacts can be traced within a short time frame with prompt issuance of quarantin .
Community containment	Intervention applied to an entire community, city or region, designed to reduce personal interactions and movements. Such interventions range from social distancing among (such as cancellation of public gatherings, school closures; working from home) to community-use of face masks to locking down entire cities or areas (cordon sanitaire)	• To reduce intermixing of unidentified infected person with non-infected community members. • It is particularly useful in settings where community transmission is substantial .

On the other hand, the responses and precautions of some countries differ to limit the spread of the Corona virus and treat patients with it, depending on the infrastructure and health systems of these countries. After the outbreak in China, then the European countries became a focus of the spread of the disease, and this has many reasons that will not be addressed in this report, but it can be summarized in the dependence of these countries on tourism and trade with other countries, which may be the reason that helped spread the virus significantly there. However, it can be noted that the European Union - although some countries have not implemented it - has a plan to confront the outbreak of any global pandemic that may threaten the public health, which can be considered to be followed in most countries and even non-Europeans, Figure 3, that is summarized in the definition of risk first, then planning and training with cooperation between all sectors [13].

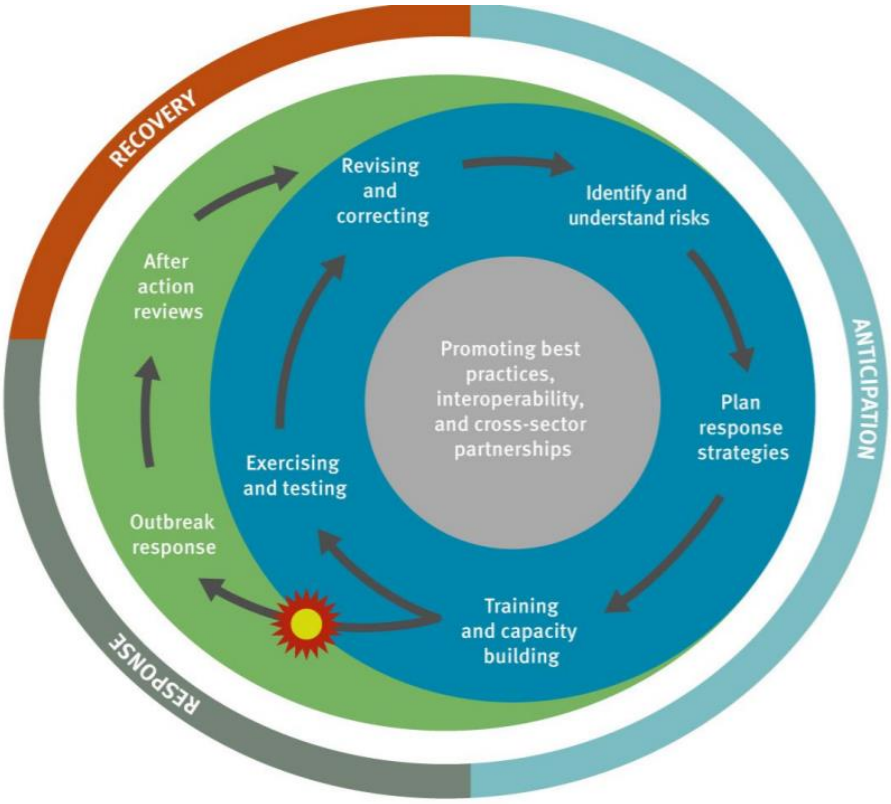

Figure 3. The preparedness cycle for pandemic s of high consequence infectious diseases (HCID).

3. BIOMEDICAL DEVICES AND TECHNOLOGIES DURING COVID-19 PANDEMIC

In diagnosis and treatment of Covid-19 disease, a number of medical equipment and devices are needed and the success on handling this pandemic is proportional to the availability of these medical equipment and devices. Personal Protective Equipment (PPE) including respirators, surgical masks, gowns, gloves, face shields, Diagnostic tests and test devices, Intensive Care Unit equipments such as beds, ventilators, and Medical diagnostic devices like Computed Tomography (CT), ultrasound device can be referred as needed medical device and equipment for fighting Covid-19.

Safe working area is provided for health workers with enough PPE. When it is provided, the number of infected health workers can be kept under control. Also, in order to reduce the number of entrances to a patient's room, catheters should be implemented when the patient comes to the intensive care unit [14]. Patient monitoring is another important point during treatment. Information such as side effects of the used medications and general situation of the patients is provided by monitoring.

While the Coronavirus most affected symptom is seen in the lungs, causing a failure in the breathing process, the ventilators are able to take over it. This gives the patient time to fight off the infection and recover. Difficulty in breathing is seen on 19% of Covid-19 patients. According to the latest data, for 5%-

14% of patients, non-invasive mechanical ventilation; for 2%-12% of patients, invasive mechanical ventilation is applied [15-17].

Showing lungs in a radiological way has a great importance. Due to the limited number of application centers and false negative results of Real-Time Polymerase Chain Reaction (RT-PCR) test, radiological imaging is used. Chest X-Ray, CT, and ultrasound are applied. CT shows focal ground-glass opacity and multifocal lung opacity in early stage (2-4 days). Then, during the middle stage (5-13 days) an increasing lung consolidation and higher rates of bilateral and multilobar involvement was shown. Late-stage CT findings (14 days or longer) showed varying degrees of clearing but no resolution up to at least 26 days [18]. According to the studies, sensitivity of RT-PCR test is 71% and sensitivity of CT is 98% in early stage of the disease [19]. Ultrasound can be used as a tool to identify Covid-19 findings such as tracking pneumonic infiltration and thickening and/or irregularities in the pleural line [20].

Role of 3D Printing Community

The number of medical devices like PPE and ventilator may not be sufficient while fighting Coronavirus and they have vital importance. A number of countries have stated their need for these devices for prevention of the disease and treatment. As demand exceeds available supplies of these devices, the supply chain is stressed. With increased number of Covid-19 cases, increased use may exceed the available supply of PPE, that results in a shortage at healthcare organizations [22]. It is aimed to combat Covid-19, and respond to the need for personal protection with 3D printer technologies. Across the globe, specialists, engineers, and designers have already begun to respond to the global crisis in order to support the manufacturing of PPE and other medical devices [23]. And also, there are calls for volunteer work where anyone who is enthusiastic to support can join the community.

Besides manufacturing masks and face shields, there have started different approaches for developing solutions to ventilator shortage problems. Multiple patients treatment with one ventilator is an important development. In the United States, a team of engineers at Johns Hopkins University was able to develop and prototype 3D printed ventilator splitter [24]. Moreover, in Italy, hospitals have run out of respiratory valves, and the original supplier was not able to meet the sudden high demand. Which was an opportunity for an engineering firm to manufacture these valves by 3D printing with an affordable price [23]. According to the demands, a 3D-printed respiratory device can be produced as a prototype in order to meet the need [25]. In addition, 3D- printed nasal swabs are in use to support Covid-19 tests [26].

3D- printed tools and components can be used instead of original components in a short time. While bringing benefit, these designs could bring risk as well. Quality, safety, and efficacy of these designs cannot be ensured unless proper quality controls and processes are done during and after manufacturing.

Artificial Intelligence Applications During the COVID-19 Pandemic

Artificial intelligence- based technologies help healthcare organizations handle viruses and fight them back. It may be used to identify potential upcoming pandemics or epidemics in earlier stages before they spread. Through analyzing data, prediction and tracking of patients is possible. It may also be used for more proper understanding of despises as well as developing and testing new vaccines.

- Early detection and diagnosis of the infection:

Artificial intelligence can be used for analyzing people's data, their internet-searching subjects for identifying and detecting irregular symptoms and how frequent they are which may be very effectively helpful in the decision-making process and thus providing a faster response to epidemics.
AI technology can also help in developing new and more global diagnosis and management systems for the epidemics. This can be through in creating new faster ways for diagnosis of cases [27].

- Monitoring the treatment and the global cases distribution:

AI can be used in analyzing the day-to day cases around the world for predict the future spreading behavior of the epidemic in order to acquire a better preparation and response which can be life-saver as well as lower costing [27]

- One great application of AI is the tracing of individuals with viruses and monitoring them which can help in identifying the circle of people that they might have infected. This can be used in minimizing the spreading of virus to a wider range of people and thus help in controlling and eliminating the disease. An example of that is Apple-Google's joint effort for notification people via mobile phones. When a person contacts someone with coronavirus, the program will notify him that someone whom he was in contact with has tested positive to the virus [28].

- Also, AI is used for drug delivery design and development for vaccines. Ai can spot patterns in data, analyze the structure of viruses, compare with previous cases, identify some possible drug prospects to be tested on humans in months which would take years in a normal way. For that to happen, a huge amount of information and researches should be obtained. Using AI allowed researchers to see and identify patterns in data that they couldn't see with the ability to analyze lots of compounds in very short time [29].

- Reducing the workload on healthcare facilities and workers during pandemics which is caused by huge numbers of patients in a short time. AI based tests can make the process of testing and diagnosing of infection in patients easier and more efficient [31]. One example is an AI-based automated CT image analysis tool for detection of coronavirus infection in patients. The system uses some previous machine learning models that were then modified for this purpose with the use of Multiple international datasets as material for the system. They said the system was able to differentiate coronavirus patients from non-patients [30].

4. VACCINE DEVELOPMENT

By May.2020 several hundreds of companies and research entities are working on developing about 115 candidate vaccines, 271 potential therapy for covid-19.

Vaccines in Clinical Trials Phase

Vaccines are made against specific functional part of the virus inactivating the virus, COVID19 disease is caused by SARS-COV-2 virus, this virus has a spherical structure, on this structure a spikes of different proteins are located, S protein is one of those proteins that binds to the ACE2 receptors in the cell allowing the diffusion into the intracellular environment.

The immunological response for SARS-COV-2 virus starts by recognising the virus by the antigen presenting cell called macrophages, macrophages will devour the virus breaking it down into small pieces, the S protein that covers the surface of the virus will be presented on the mhcII molecules stimulating the T cells that will leads to activating the B cells that has the receptors of S protein which is lymphocytes, B cells then copy itself and turn into a plasmid releasing antibodies.

The response of the immune system for SARS-COV-2 is not immediate and may takes around one to two weeks, during this time the proliferation of the virus may causes death or sever statues, the vaccine role is to introduce the antigen with non harmful way, then by activating B cells that save a copy of the antigen, quicker response will occur when the real virus attacks.

However, the virus will dispersal around the world and by moving around nations will be exposed to genomic mutation, the vaccine designed should keep effective till with mutated virus [32].
There are different types of vaccines under research, below is a quick review for the ones that entered the clinical trials stage:

DNA Based Vaccines:

- DNA plasmid; INO-4800

DNA plasmid is a double strand DNA (normally taken from bacteria) that has the property of being programmable by computer sequencing technology, DNA plasmid is used to deliver an optimised DNA into the cell to induce specific immunological response generating a robust targeted T cells and antibody responses, for this candidate vaccine a device called CELLECTRA® 2000 used to provide and electrical pulse that allow the cells to open reversely allowing the plasmid to enter the cells. [33] [34]

Inovio Pharmaceuticals started the preclinical research by January 2020 conducting the in vivo and in vitro primary research, the clinical trials on the INO-4800 potential vaccine started by April 2020, and expected to get the initial result by June 2020 to proceed to the phase 2/3 by summer 2020 [33].

A DNA plasmid based vaccine is promising due to being cheap, safe, and big quantities can be manufactured easily which will help deliver the vaccines if they succeed for as many people as possible [35].

mRNA Based Vaccines

mRNA is a single strand molecule that transfers the DNA instruction in the nucleus to the ribosome where the proteins which are doing different functions are translated.
mRNA based vaccines can be used to induce the immunological response in the cell, by using the host cells' transcription pathway.

- 3 LNP-mRNA (RNA; mRNA; BNT162)

Researchers genetically synthesized an mRNA sequence based on the genetic information of (S) protein (that cover the majority surface of the virus[38]) SARS-COV-2, the mRNA sequence was encapsulated within a lipid nano-particle to help fusion into the cell, once the mRNA sequence is delivered into the cell

the sequence will translate the order of producing (S) protein on the cell surface which will induce the production of antibodies and therefore strengthen the immunological response of the cell. [38][37]
A collaboration between BioNTech, Fosun Pharma and Pfizer is working on developing 4 versions of mRNA vaccines some of them are in the clinical trials in phase I and other in clinical trials II, the initial data is expected by the end of June 2020. [36]

- LNP-encapsulated mRNA (mRNA 1273)

Another version of mRNA vaccine developed by synthesizing the mRNA from the genomic material of the virus's S protein gen, the synthesized will be delivered by a lipid nanoparticle into the body where it will diffuse into the cell, and the mRNA is translated into S proteins, the antigen of S proteins are recognized by the immune system inducing the production of antibodies and saving the antigen in the B cells allowing quicker response for the virus attack [38].

Research is conducted by a collaboration between three companies Moderna, NIAID and Lonza, and recently they announced the success of the phase I of the clinical trials done on 8 persons, they will proceed to phase II as they got the approval of FDA [36].

Inactivated Virus Based Vaccines

In this type of vaccines the virus is inactivated by a denaturing it using a chemical or physical factors, after the virus loses its ability of making any damage to the human body it is injected into the human body so the immune system get trained by responding for an inactive virus, and when the real virus attack best immunological response can be presented.

There are three inactivated virus based vaccines currently studied in the clinical trials.

- The Chinese biopharmaceutical in a collaboration with a company works on developing Inactivated virus based vaccines called (Picovacc) that proved efficacy in macaques, currently Picovacc is in phase 1/2 of clinical trials. [36]

- Sinopharm Group (Wuhan Institute of Biological Products) and Chinese Academy of Sciences (Wuhan Institute of Virology) are working on an inactivated virus based vaccine, they got the approval of the China's National Medical Products Administration for phase I and phase II of their candidate drug. [39]

- Sinopharm Group (China National Biotech Group, Beijing Institute of Biological Products) in collaboration with the Chinese Center for Disease Control and Prevention are working on another inactivated virus based vaccine, and they were able to get the consent for the clinical trials from the China's National Medical Products Administration for phase I of clinical trials. [40]

Non Replicating Viral Vector Based Vaccines

- Adenovirus Type 5 Vector (Ad5-nCoV)

For this vaccine, a recombination of novel coronavirus disease vaccine incorporating the Adenovirus Type 5 Vector (Ad5-nCoV), taking the gene of the S protein and inject it into the adenovirus, then injecting the adenovirus into the human body allowing it to replicate and develop an immunological response against the S protein so when the real virus activated virus attack better response of the immune system will be achieved [41].

CanSino collaborating with "China's Beijing Institute of Biotechnology and Academy of Military Medical Sciences", are working on developing this vaccine, and by April 12 Phase II clinical has been Launched [42].

- ChAdOx1

Oxford University with a collaboration with Jenner Institute is working on a vaccine called ChAdOx1 which is currently in phase I, the idea of the vaccine is to take the adenovirus from the chimpanzee that do not harm human being and treat it so it no longer has any harmful effect, then inserting the gene of the S protein in it, then injecting the vaccine in the body, when S protein is formed inside the body in safe way, the macrophages recognize it inducing the immune system to empower its own protection mechanism against the real virus when attacks.

Recently in 18 May the failure of this vaccine was announced due to its inability to resist the virus in animals.

Treatments in the Clinical Trials Phase

Corticosteroids

Corticosteroids is a repurposed drug approved by FDA since the 1950s to treat many diseases, including anti-inflammatory conditions and some cancers [36], currently in the phase III clinical trials [43].

In February 2020 a study told that no evidence support clinical treatment of SARS-COV and MERS-COV viruses by corticosteroid and referred to that it reduced the rate inflammatory response that may damage our body by the over functioning of immune system but delayed the clearance of the virus and caused complications during and after the disease such as diabetes, psychosis, avascular necrosis in survivors, Increased mortality expecting that that it will not benefit in treating SARS-COV-2 virus[44].

However a study conducted on 70 patients with COVID-19 severe pneumonia in France between 10 March and 9 April, 202 shew that a risk difference of -47.1% (95% confidence interval -71.8% to -22.5%) lowered the risk of intubation by corticotherapy, and it expected that this can be an important tool in managing severe Covid-19 patients suffer from respiratory failure [45].

Remdesivir

Remdesivir (RDV) is another potential treatment under repurposing research, previously was used as treatment for EBOLA, but still does not have the FDA approval. This research is in the phase III of clinical trials, and runs through a collaboration of three pharmaceutical entities, GLIAD, NIAD, and Feinstein Institutes. [36]

Study has been conducted on RDV efficacy on MERS-COV explained how RDV can be an effective treatment candidate against SARS-COV-2, the mechanism of action is that after the virus bind to ACE II receptor and fuse into the cell, the virus RNA is translated into different proteins one of them is RDRP, this protein the reverse transcription to the mRNA increasing the load of the virus, RDV works by mimicking the nucleotide base of Uracil (U) that places on the virus complementary mRNA preventing the right replication of the virus RNA, leading to reduction in both virus load and damage to the immunological response by reducing inflammation [46].

Another recent study showed that RDV enhances the recovery and accelerates the discharge of COVID19 patients, lowering the length of stay of hospitalization by a half, and reducing the death from COVID19 by 29% compared with those of the same severity rate with no RDV treatment [47].

Chloroquine and Hydroxychloroquine

Chloroquine and Hydroxychloroquine are antimalarial drugs that are under repurposing research for using for Covid19 patients, those two treatments has been issued by FDA for the emergency use by 28 march 2020, where there are over 50 clinical trials registered at clinical trials.gov [36].

The main property of these candidate treatments is their weak basic nature, knowing that the intracellular is an acidic environment, and the different enzymes function inside the cell need this acidic environment we can expect the role of those treatments. Decreasing the acidity of the intracellular environment of the cell will decrease the virus ability to diffuse into the cell, and the part which may be diffused will be so difficult to reassemble and replicate due to the need of the less efficiency of the intracellular machinery in basic environment.

Another benefit aspect of the hydroxychloroquine is being Zinc ionophore, meaning it help forming a channels to move the Zn++ through the cells membrane as by nature the cell membrane is not permeable to Zn++, the importance of the Zn++ is that it disables the function of RNA dependent RNA polymerise needed for the replication of the virus inside the cell.

Hydroxychloroquine decreases the efficacy of the SARS-COV-2 receptors (ACE II) by preventing or reducing the glycation needed for the last amino acid of it during receptor building in the cell.

There are tons of studies that prove the efficacy of the inefficacy of the hydroxychloroquine that need special work to collect and summarize, however most of them are conducted on a small group of patients, and the results achieved depend basically on the patient profile and the administration way.

Favipiravir

Favipiravir is an antiviral drug licence in Japan to treat influenza, clinical trials on repurposing this treatment is conduction in Japan (phase III), USA(II) and India phase (III) with an expected results by July/ august [36].

Favipiravir function by inhibiting the RNA polymerase of RNA virus, blocking the replication of the virus [48].

Effects of Vitamins and Minerals

Vitamin C

Vitamin C has an antioxidant property that is important for neutralizing reactive oxygen species, Vitamin C is needed for H2O2 formation in the immunological cells to respond properly during.

China is conducting a clinical trials on the effect of the IV administered over dose of vitamin C for the treatment of sever Covid19 patients [49].

Vitamin D

Vitamin D is essential not for treating or inhibiting the virus but to keep the immune system works better, vitamin D has a role is suppressing the innate immune response and balancing the proinflammatory acquired immune response by modulate the releasing the Cathelicidin, Beta defensin and NFKB [50].

Recent studies showed that vitamin D deficiency is a reason for C reactive protein (CRP) increasing rate, “CRP is an acute-phase protein binds to the dead or dying cells in order to activate the complement system” [51], Increasing the CRP rate will decreases the innate immune response which will rise the viral load and cause the severe immune response which may demand the patient to enter the ICU [52].

Zinc

Recent study has been mentioned that using the Zinc with hydroxychloroquine would affect the people discharge home number and would not effective on the length people being in ICU or get ventilated, Zinc play in important role in decreasing the replication rate of the virus RNA when it delivered to inside the cell by hydroxychloroquine [53].

5. CONCLUSION AND FUTURE PERSPECTIVES

Our world is currently facing the greatest challenges at all levels in the century; the novel Coronavirus, as these challenges were not limited to health problems only, but also to social life, the economy, unemployment rates, tourism and many others. Very few countries and places have relatively succeeded in confronting this disease and limiting its spread by taking the necessary measures and the effective use of resources. But, on the other side, the outbreak of this disease began very quickly, which did not allow many other countries to confront its consequences, as the shortage of medical devices and personal protective equipment puts many people, especially doctors and health workers, in inevitable danger. However, compared to the previous epidemics that humankind has undergone, there is no doubt that the development and technology has great impact in containing this disease, such as the use of 3D printing in the manufacture of ventilators and personal protective equipments, in addition to the use of artificial intelligence in the diagnosis and follow-up of patients to treat them, monitor their health and narrow the circle of infection.

Till now, there have been many advances in the development of a cure or vaccine for this virus. Some have been shown to be effective such as corticotherapy, RDV, Chloroquine, Hydroxychloroquine and Favipiravir, which are still in different phases of clinical trials, and/or issued for emergency use only. However, what is frightening in the new Corona, despite the research that has been carried out, is that researchers are unable to understand the mechanism by which the disease is transmitted from animal to human and thus weak ability to control it. Not to mention the high prevalence among people, the high mortality rate among critical groups and the lack of completely curative treatment so far.

Although we cannot predict precisely what days hide for us, we believe that this cloud will disappear one day, by our awareness, our unity, and by following health measures; we can triumph over this disease with the least possible losses.

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The Importance of Nano Biosensors and Ethical Elements in Sports Performance Analysis

Review Article

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Abstract

Performance analysis is an indispensable element for elite sports success. Coaches and teams are constantly striving for ways to improve and maximize their performance. Effective use of performance analysis enables tactical, decision making, better athletic confidence and improvement of reflective coaching technique. Performance analysis is required for coaches and athletes to ensure consistent success rates, especially at elite levels. Although performance analysis seems necessary in elite sports, we should recognize the benefits and disadvantages associated with the individual athlete and sports system that regulate their distribution for their use. These are a new technology, nanobiosensor, a concept that combines biomedical field with sports engineering and nanotechnology and ethically addresses future performance analysis in sports. In this study, this concept is discussed. This innovative technology has the potential to revolutionize sports and enables athletes to collect real-time biological data electronically. Affinity-based nanobiosensors have also been taken into consideration in sports medicine and doping control analysis because they are cheap and easy-to-use, yet selectively analytical devices. Allows the use of the same sensor for multiple analyzes. At the same time, nanobiosensors can contribute to filling an important information gap about complementary evidence from their “on-site” use and pre-selecting the risk population of individuals to be targeted for a complete antidoping test; In sports medicine, they can contribute to obtaining analytical knowledge of physiological relevance from measurement of specific parameters or markers before, during and after physical exercise. However, as with many technologies, this technology may have undesirable uses. These unwanted uses are data ownership and privacy may affect data privacy and well-being of athletes. While the use of nanobiosensors in sports analysis in the future offers many potential benefits, there is also a concern that they can be abused. For this reason, it is essential for sports organizations to consider the development of a sound, ethically informed governance framework before

their increased use. Thus, the value of sports and athletes will become more prominent. For this reason, it is very important for athletes and their regulators to develop measures to ethically integrate this technology into sports.

Keywords: Sports, Performance Analysis, Sports Engineering, Nanobiosensors, Ethic

1. INTRODUCTION

According to the British Sports Institute, the discipline of performance analysis focuses on improving interventions in the coaching process, allowing performance gains and increasing learning. The discipline of performance analysis stems from the desire to make good performance gains by improving tactics and techniques through feedback. The most important step of the coaching process is to get feedback about the player or team in order to increase the performance of the player and the team (Nic, 2009). Therefore, objective measuring tools are necessary to enable and facilitate the feedback process.Sports Performance Analysis gives coaches objective information that is used to optimize team and player performances. Research has shown that athletes and coaching teams contribute only 30% of the performance performed by an elite athlete on average, which can only lead to minor gains for the athlete. The remaining 70 percent should be supported by performance analysis (Evans et al., 2017). Performance analysis is not homogeneous. It can be performed according to location, timing, biological and psychological differences. For example, it can take place after the performance of an athlete, or laboratories that provide a more controlled environment. There are several ways in which performance analysis techniques can be used in sports. For example, using visual feedback can be the use of the athlete's profile data to develop a game plan ready to compete successfully in training in a competitive environment. Performance analysts use advanced video performance software systems such as 'Dartfish' to provide this type of information (Dartfish N.D). The increasing sophistication, and reducing cost, of video systems have greatly enhanced post-event feedback, from playback with subjective analysis by a coach to a detailed objective analysis by means of notation systems (Brown and Hughes, 1995). These systems provide athletic advances with advanced analytics that encourage more critical performance feedback and interaction between coaches and athletes.

1.1.Benefits and Losses of Performance Analysis

Effective use of performance analysis enables tactical / decision making, improved athletic safety, and improved reflective coaching. Performance analysis is required for coaches and athletes to ensure consistent success rates, especially at elite levels. Performance analysis can be a very useful tool when used to monitor and feedback team skills, strengths, and weaknesses. Coaches or analysts can keep statistics about their team and the opposing team. He can make comparisons as to which side does what better and why he performs better. Performance analysis can also be a very useful tool when used to monitor individual players' skills, and their ability to carry out game-plans and strategies. Stats can be produced to point out the player's strengths and weaknesses, which is essential when optimizing performances (Taylor, 2017). Although performance analysis may seem necessary in elite sports, we should recognize the advantages and disadvantages associated with the individual athletes and sports systems that regulate their distribution for their use (Table. 1).

Table 1. Advantages and disadvantages to both the athlete and the sports system

Advantages to the Athlete	**Advantages to the System**	**Disadvantages to the Athlete**	**Disadvantages to the System**
Performance data increases.	It provides, safety in sports	It harms athlete's consent, confidentiality and data ownership.	With technology doping, it creates unfair competition.
It creates added value for the athlete.	It discourages cheating.	It makes the athlete unskilled.	Facilitates corruption / decay.

Recent advances in bioengineering are essential for performance analysis. The development of sensor and image processing and feedback systems enables analysis by transferring data in a digital environment. This has enabled much more effective feedback systems to be developed with increased data levels to be achieved in less time (Baca et al., 2006). If we look at how performance analysis and feedback systems are used in swimming sports, swimmers of the Great Britain National Team can be an example of this system. The way the system works is to follow a swimmer from the water wirelessly (Epsrc, 2012) to ensure that all movements of the athlete (such as body position, acceleration, and general technique) are tracked. The information obtained is transmitted to analysts and coaches who can provide feedback to make real-time adjustments during training. Then the coaching team should provide feedback to swimmers through observers and post-video training, but this can sometimes result in loss of precision and time. Despite these benefits of the system, it is limited as it only focuses on the external movements of an athlete. For instance, to learn about the relative fatigue of athletes, should be given a lactate test and blood samples and such data should be analyzed far from the field of training as should be taken in mid-training. As a result, sports engineers have explored other areas of research such as biomedical and engineering to solve this problem. As a solution, they developed biosensors to capture biological data via mobile devices. Some sports models use wearable technology to calculate and record basic physiological data and instant performance data. (Smith, I. A. 2014). Biosensors can coach athletes and wirelessly collect performance data such as athlete's acceleration position, respiratory rates, and fatigue levels. It allows continuous monitoring of an athlete (Moskvitch, K. 2012). In this way, the physical condition of the athlete can be followed more extensively and gives more information about how the body parts look like during training and competition. Also, data collected with biosensors can be used to predict what could put an athlete's health at risk, thereby promoting early diagnosis of conditions such as cardiac arrest, making the sport potentially safer for the athlete (Moskvitch, K. 2012). In summary, we can say that the health profiles of biosensors and athletes can be monitored completely. However, we should ask more precisely which biosensors are and how they can be applied in elite sports.

1.2.Feedback Systems: Biosensors / Nanobiosensors Performance Analysis Systems

Before dealing with the ethical aspect of nanobiosensors, we should think about what the biosensor is. It describes the Higson biosensor as "a chemical sensing device in which the presence of a biologically derived recognition is connected to a transformer to enable numerical development of some complex biochemical parameters" (Higson et al., 1994). Fraser, on the other hand, defines it as an "analytical device

containing a deliberate and intimate combination of specific biological elements and physical elements" (Fraser., 1994). Biosensors consist of a bio element and a sensing element (Mohanty et al., 2006). Using a biosensor, an athlete's performance can be maximized because it can keep athletes at their highest physical performance levels. However, it is very difficult to stay in this state when exercising at the extreme for a long time. It is often said that the health condition is good where the elite condition starts. Therefore, precise, accurate and timely monitoring is very important for athlete-centered sports technology and sports medicine (Dijkstra et al., 2014). An example of a biosensor used for sports is a Pulse oximeter (Sheehan, K. 2010). This is a hand-held electronic device used to measure the amount of oxygen in the blood of athletes. It emits infrared light by plugging and running on an athlete's forefinger. Oxygenated blood absorbs the blood of athletes at different levels, which allows the calculation of the correct oxygen level (Montgomery, 2010). Pulse Oximeter is used to monitor potential oxygen drops during such situations and allows coaches and athletes to develop new methods to increase endurance for higher competitive gains. Given the change in the lactate concentration in the body fluids of the athletes during physical activity, the lactate test is a test that determines the condition and endurance capacity of the athlete during the exercise by monitoring the lactic acid level. The most commonly used method for determining the form of individuals and determining individual training weight is the lactate test. The barcode system obtained to make measurements during exercise is integrated into the adhesive tape, sportswear, sweatbands for the waist and head as seen in Figure 1 (Martinez, A.W. 2011).

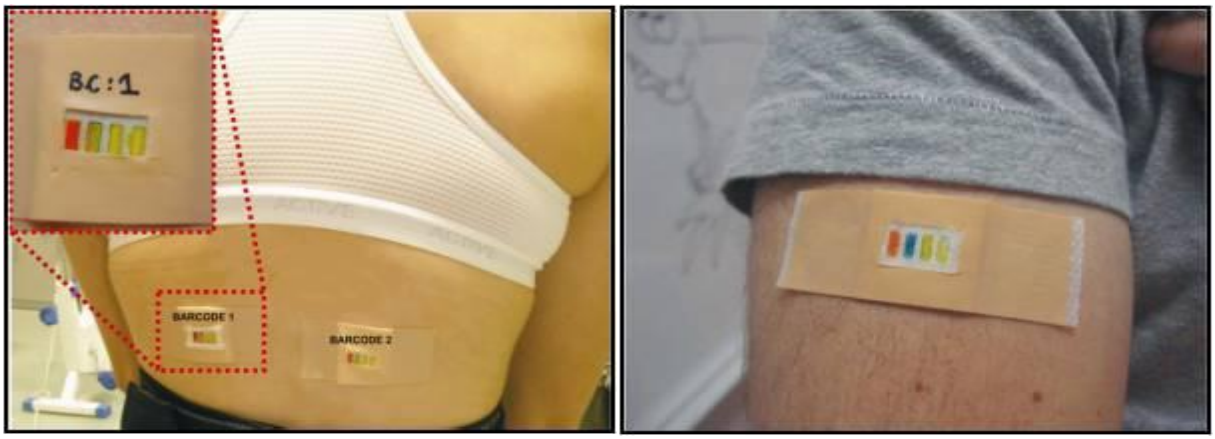

Figure 1. Barcode systems on the athlete's back and arm

The use of such sensors for sweat analysis provides valuable physiological information for applications in sports performance (Coyle et al., 2009). RBC Life Sciences®, Inc. The "Nanoceuticals ™ Slim Shake Chocolate" produced by the product contains nanoparticles that clean free radicals more effectively, increases hydration, regulates body pH, reduces the amount of lactic acid formed during sports, and reduces the surface tension (www.nanotechproject.org). However, the volume of biological data that biosensors (1) can collect; (2) practical functionality in terms of size and body restriction for elite athletes; and (3) they have extreme sensitivity, which can sometimes lead to incorrect data measurement. As a result, sports and biomedical engineers tried to overcome these deficiencies with nanobiosensors. Nanobiosensors generally differ from biosensors only in scale. A nano biosensor can be defined as a biosensor consisting of nanomaterials and dimensions on the nanometer (Atta et al., 2011). The incredibly small nanobiosensors have many advantages due to the large surface area/volume ratio. Many nanomaterial atoms are located

near their surface, which leads to improved transduction and signal capabilities (Malik et al., 2013). This increases their ability to detect and provide more accurate data logging. Also, these devices can be installed on a person for a long time without irritation.

1.3.Analysis of Performance Feedback Systems as an Example of Nanobiosensors

Nanobiosensors offer enormous potential for elite athletes and are at the stage of research, with teams around the world developing and trying new ways to use them in the elite world of sports and medicine. Nanotechnology engineers have developed a Nanotattoo that can be placed on an athlete's arm to monitor lactate sweating levels (Jia et al., 2013). Lactate monitoring is an important indicator for evaluating physical performance during training and competition with multiple sprints and speed resistance. Traditionally, lactate levels have been monitored using lactate sensor strips used with a hand-held device that is both convenient and uncomfortable during physical activity (Jia et al., 2013). In contrast, the non-invasive enzymatic temporary transfer tattoo acts as a flexible sensor made by screen printing methods bent to fit the body. Determining more precise measures of athlete lactate levels, which is a significant performance barrier, allows significant improvements for athletes and coaches; because it enables the immediate acquisition of data on biological performance, allowing immediate intervention in training sessions. Let's look at the advantages of some nano biosensors and biomarkers. Corner technique is used to evaluate the training data of boxers. Thanks to the three-axis accelerometer on the produced glove, it is possible to measure the characteristics of athletes such as stroke forces, stroke combinations, stroke rates, and blocking times. This ensures that the data of the athlete is stored during training and the performance of the athletes is improved. Goal post-integration using sensor fusion has recently been implemented on two technical bases to scan a specific area. The first one is based on the cameras installed in the stadium structure, and at the same time, it makes a decision based on the position of the ball relative to the target line based on the view of at least three different cameras simultaneously. The second technique is based on magnetic field sensors placed on three goalposts, where the decision will be made based on the magnetic field change. Both techniques process the signals from the sensors and transmit them to the referees via wireless encrypted communication (Shan, P., 2014). To investigate the physical performance of an athlete on the field, a GPS (Global Positioning System) is used to collect data such as speed, position, acceleration, time of each activity type in each player. Therefore, the use of sensor fusion for football analysis is essential for rule enforcement and monitoring and evaluation of players (Kocakulak, 2019). Another sensor application is designed for athletes struggling in basketball and baseball, and the interactive armband can be used to determine the beat and shot rhythms of the athlete and to create a muscle rhythm map. The proposed basketball instruments were developed using nine accelerometers placed on the ball that communicate with mobile devices and allow the user to receive data through an application installed on the device. This type of analysis helps correct movement and shots to improve the quality and accuracy of field targets (Kocakulak, 2019). “Readiband" electronic tapes designed for athletes can also measure how much sleepers sleep at night and sleep quality. Thanks to the accelerometer in the band, it detects whether the athlete is awake by measuring the movements of her wrist. hanks to the SAFTE (Sleep, Activity, Fatigue, Task, and Activity) module, a sleep quality score indicating the sleep quality of the person is calculated.

Let's look at some ethical and legal concerns before nano biosensors are widely applied.

1.4.Benefits to the Athlete

Higher Value Performance Data:

Previously, performance analysis was limited due to the inability to prepare biological data for analysis. However, with the integration of biosensors and nanobiosensors, coaches and athletes began to better understand how an athlete's body works. Thanks to the future use of nanobiosensors in the form of wearable technology, a range of bodily functions, dehydration, healing, lactate levels, and even wound healing rates can be tracked wirelessly will present the picture (Ray et al., 2019). In addition, nanobiosensors increase the gather speed and usability of the data, providing emergency interventions to coaches and athletes with data transmitted in real time on their smartphone or tablet. Wearable microfluids touch the human skin directly with wearable technology, so that data can be analyzed by wireless data transfer (Kocakulak et al., 2019).

If we exemplify the performance analysis data measurement with the football branch; GPSPORTS training vest technology is also widely preferred for team sports today, while it is a smart sensor especially used in football branch. Arrangement of training data and stations can be made with the vest design that allows the measurement of the movements, body activity and performance data of the athletes in the team (Akçalı., 2016).

Figure 2. GP SPORTS training vest technology (Akçalı. 2016).

Increased value for the athlete:

In today's modern sports world, statistics based on the performance of the players during the season have become very important for economic contract negotiations. High-performance data can provide contract opportunities for athletes and coaches. Recent developments in biosensors have provided significant improvements in the study of athletes' biological data, along with indicators of future injury rates for athletes in the long term. Strong biometric data positively affect an athlete and club. It also increases the economic value of the player as well as the success and economy of the club. Other measures focus on the lactate production level, heartbeat, but nano biosensor is used to further justify data readings. It can make

direct comparisons between players' biological data. This can also be advantageous for clubs, as it can provide coaches and managers with data that can rely on depositing quality players without fear of return due to long-term injury.

1.5.Disadvantages of the Athlete

The qualification of the athlete:

The more general use of engineering in sports can render athletes unskilled, more confident in the technology (Hunter., 2011; Miah., 2006). The athlete does not want to become unskilled. The use of technological innovation has made performance comparisons of many athletes less meaningful. The role of sports engineering is to support the development of an athlete. Therefore, it is essential to use nanobiosensors to support the athlete rather than eliminating the skill level required to become an elite athlete (Carr., 2008).

Consent, Privacy and Data Ownership**:**

The current use of biosensors and the use of future nanobiosensors for performance raises serious concerns surrounding the collection of biological data. Despite the benefits that nanobiosensors may bring to the performance of an athlete in the future, it should be carefully balanced against key issues such as privacy, security, consent, and data ownership (Meingast et al., 2006). The use of nanobiosensors in sports requires clear and controlled regulation. Even doctors take the consent of his/her patient and tell him about the risks of what he/she will do before taking any action (Kegley., 2004). Also, the data of athletes using this nano can pass to third parties at once (Meingast et al., 2006). The use and storage of data that could harm the interests of individual athletes is an issue that needs to be addressed ethically. The questions of who owns such data and who owns related information flows are important. There are serious concerns about privacy and security regarding the use of nanobiosensors. The data collected and stored by the athletes can be captured by hacking, and this can give other teams an advantage. Also, for example, the physiological life signals of athletes are sensitive and can determine that they have an embarrassing illness or a career-ending situation, so leaking this information undermines the privacy (Kumar et al., 2012). The constant use of nanobiosensors raises concern about the blurring of the private / public distinction. 24/7 monitoring of athletes again undermines the privacy of private life.

1.6.Benefits to the Sports System

Improves Safety in Sports:

Sports engineering increases safety in sports. This can be exemplified by the development of wearable items, from clothing to shoes. The growth of nanobiosensors in the future may provide more safety in sports. The use of biosensors can reveal a safer playground, help prevent injuries, ensure that athletes comply with vital health data to risk standards (Evans et al., 2017). With the use of such biosensors, injuries and increased sensitivity are prevented by creating a safer playground. It can be used to control vital health data and ensures that athletes comply with agreed risk standards.

During the match played with the developed Checklight technique, the severity of the pulses taken is measured in order to control the blows to the head area and to protect the athlete's health. This product can be measured in the sports branches (ice hockey, American football), where the physical contact is intensely carried out, and the severity of the blows received with a helmet placed inside the helmet. Thus, the first intervention is performed healthier and the health of the athletes can be protected according to the severity of the blow received (Akçalı., 2016).

Figure 3. Check light technology product example (Akçalı., 2016).

Therefore, this new technology is not only limited to the performance analysis required in sports, it is also very important for the athlete's safety in order to prevent long-term injuries.

Prevents cheating:

In the current system, some drugs can be saved from doping tests, that is, they are not caught. Moreover, the application of micro doping made it extremely difficult to detect doping. Increasing the development and use of biosensors and nanobiosensors can bring a better application with regulator balance thanks to increased sensitivity to cheating. Biosensors can be integrated into wearable microfluidic chips to meet different requirements of testing body values with fewer technical barriers (Yi-Qiang et al., 2017). With the Athletes' Biological Passport, the athletes can be tracked even while on holiday and checked whether they cheat (MacGregor et al., 2013). Nanobiosensors can be used to further develop this feature (continuous monitoring) due to greater sensitivity and more real-time tracking potential. This can be very useful in increasing confidence in sports, especially in sports such as athletics and cycling, where doping scandals undermine sports' reputation and commercial value.

1.7.Disadvantages of the Sporting System

Unfair Competition in Sport: "Technology Doping":

Technology doping is a new concept. It refers to giving an athlete an advantage by using technology. After all, sports are a competitive business and, for example, takes the lead if the British cycling team can produce better bicycles than in other countries. Or financial fair play aims to balance the spending on

football teams and avoid unfair competition. Concerning nanobiosensors, the issue of technology in justice and sports can also be brought up. We have mentioned that the technology offers athletes to access higher levels of biological data for performance analysis. We can reasonably assume that not all athletes can access these sensors due to the high patent costs; It is an unfair advantage for those who have those sensors. For example, during a marathon or bike race, athletes can use Nanobiofeedback to correct their tactics. The coaching team may notice, for example, that the athlete's glucose level is decreasing, and therefore advise the athlete to take glucose as soon as possible to maintain consistent energy levels that can determine winning or to lose. Therefore, deciding when to run a marathon runner, thanks to nano biosensors, potentially creates unfair competition. There is also a concern that sports can be a test of the power of technology systems, not a test of athletes' abilities.

Corruption / corruption:

Using nano biosensors for performance management provides many potential benefits, as mentioned earlier, but their use in elite sports also has the potential to increase corruption in sport. Providing real-time transmission of the biological data of an athlete or team equipped with nano biosensors with WI-FI in the future may result in exposure to data piracy by deliberate theft or leakage of important information to competitors. If doping is the biggest threat to the integrity of the sport, match tuning and handicapping is the second. We can count the good properties of nano's, but they can also bring corruption in sport. It is quite possible scenarios that the data of the team and players are stolen or that someone inside leaks to the opponent team. Spending on this way, the opposing team gains knowledge of the opponent's tactics and all kinds of in-field situations of key players. Stealing biological data is also effective in the match setting. While it is ensured that nano biosensors are added to the elite sports, it is very important that the relevant institutions meet the potential losses that may arise due to this technology.

2. CURRENT AND POTENTIAL SOLUTIONS TO ETHICAL PROBLEMS OF NANOBIOSENSOS IN SPORTS

An athlete may sign legal documents to ensure that their biological data is protected from abuse, properly stored, and not used against them. There are regulatory institutions on such issues related to consent. One of the most important future developments in the integration of nanosensors is the development of a regulatory framework and policy for their use in sports. A regulation set will protect both the athlete and the system against contamination. Although regulators exist to protect an athlete's data and privacy, concerns remain about privacy and consent. It is therefore important that sports organizers work together to develop a governance framework that can be applied when preparing for this technology. A regulatory framework will be required that sets limits on how these sensors are used in athletes' private lives. We can predict the 7/24 data collection cycle to take advantage of the nano biosensor potential. This will increase even more during peak competition times but will collect performance data that is not important even at rest. Also, a well-planned security network must be created and used to protect the wireless network where nano biosensors will work without potential hacking, and to protect the biological data of athletes, to ensure privacy and confidentiality (Kumar et al., 2012). This will allow athletes to have more confidence in using nano biosensors and therefore will be more willing to use. Consequently, these are key elements that need to be discovered and developed before their integration into elite sports and to

ensure that all future concerns are fully evaluated. It is essential to take precautionary measures at any intermediate stage to promote the safe and ethical use of nanosensors for both athlete's well-being and the elite sports system as a whole. This will also help raise the ethical profile of technological development in sports, and thus will be seen as an essential element of sports management. Despite all these data, nanosensors are not yet used in a wide range of sports. Because it is a very expensive and difficult technology, it cannot be used by everyone. According to some sports circles, this situation creates unfair competition. For example, the International Federation of Swimming (FINA) banned nanotechnology-supported swimsuits as "technology doping" in 2010 on the grounds that it created unfair competition. Regardless of the sports branch realized in such cases, athletes or teams that are stronger in monetary terms are thought to gain an advantage to their rivals thanks to this technology. Athletes with limited abilities are thought to perform better with the help of nanotechnology products (Kocakulak, 2019; MacGregor, 2013). In order to eliminate this negative situation, it may be an alternative solution to pull the nano-supported products to the levels that everyone can reach in the field of sports and thus prevent unfair competition that may occur.

3. CONCLUSIONS

Performance analysis is an important training and competition tool for every elite athlete and ensures that they continue to achieve basic marginal gains through reflective feedback practices. Performance analysis is also a constantly evolving practice that includes areas such as sports engineering and biomedical research, which use the latest technology to get more analysis and feedback from athletes. The integration of biosensors in sports performance analysis has enabled the acquisition of biological data for new levels of feedback about the performance of athletes in both training and competition. This integration has begun to provide significant benefits to athletes in the world of sports, and with the future developments of nanobiosensors, all of these are likely to improve further. However, before nanobiosensors become a daily tool in sport performance analysis and development, regulatory agencies should be taken into account in order to take into account the points in which access to data is not taken into account - data access, ownership, privacy, privacy as well as athlete well-being. Thus, the value of sports and athletes will become more prominent. It is therefore important to develop measures for sports organizations and regulators to ethically integrate this technology into sports.

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Viability and Morphology of Human Dental Pulp Stem Cells in The Presence of Citrus Pectin

Research Article

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Abstract

Pectin is a galacturonic acid rich heteropolysaccharide which regulates plant cell metabolism. Plenty of fresh fruits and fruit pomaces from fruit juice production can be used as a raw material in commercial pectin production. Pectin occupies a large global market size especially in food industry and the utilization of waste materials for obtaining pectin molecules as a high value-added product makes it very favorite industrial material. Besides food industry, pectin is gaining attention in tissue engineering and drug development studies. In this study, the effects of citrus pectin on viability and morphology of human dental pulp stem cell (hDPSC) were investigated. The cells were cultured in the presence of pectin in culture medium (0.43, 0.85 and 1.7 mg/mL) for eight days. Resazurin application and MTT assay were applied on day one and eight for cellular viability. Cellular morphology was investigated by invert phase contrast microscope, live/dead cell staining and F-actin/nucleus immunofluorescence staining. MTT analysis results indicated that the viability of hDPSCs decreased significantly due to dissolved pectin in culture medium at applied concentrations. There was no significant morphological difference in the cells under invert phase contrast microscope and no significant staining difference in live/dead cell staining images. On the other hand, F-actin/nucleus staining showed that there were some condensed and crescent cell nuclei in the pectin applied groups when compared to the control groups which may be related to apoptosis. In conclusion, the viability of hDPSCs decreased and crescent cell nuclei formation was observed due to the presence of citrus pectin in the cell culture medium.
Keywords: Pectin, Human Dental Pulp, Mesenchymal Stem Cell

1. INTRODUCTION

Pectin is a complex plant cell wall polysaccharide which is responsible for cell growth, differentiation and defense mechanism. Pectins, as a biomacromolecule, mainly consist of galactronic acid and rhamnogalacturanon I and substituted by rhamnogalacturonan II (RG-II), and xylogalacturonan (XGA) [1, 2]. They are divided in two groups according to the degree of esterification. High methoxy (HM) pectins

have esterification degrees above 50% and and low methoxy (LM) pectins have esterification degrees below 50% [3-5].

Pectin is a widely used stabilizer and gelling agent in food industry and has expanding market share in cosmetics and pharmaceuticals [6]. It is obtained from various sources as citrus peel (85%), apple, sour cherry pomace and black cherry tomato [7-11]. The main pectin extraction method is based on acid hydrolysis in hot water [1]. There are some examples for different types of pectin extractions: pectin extraction from grapefruit (Citrus paradisi Macf.) peel with array-induced voltage approach [12], pectin extraction from walnut processing waste with ultrasound-assist, high-viscosity apple peel pectin extraction with citric acid [13], pectin and phenolic compounds from sour cherry pomace (SCP) with microwave-assisted approach [9] and also pectin extraction from orange peel by a combination of surfactant and microwave-assisted extraction methods [14]. Especially pectin extraction from the apple pomace is easily processible and safely utilized in industrial processes and also eco-friendly method [3, 4].

The effects of dietary pectin or modified citrus pectin have been studied in the literature and it was concluded that pectin rich diet can reduce blood cholesterol, supports immune system and decreases tumorigenic activity [15-17]. In a study, increased number of surviving stem cells in intestine of mice which were fed with pectin rich diet and exposed to total-body gamma-IR was showed. This result indicates a radioprotective property of dietary citrus pectin [18]. Pectin extracted from cacao pod husks after deacetylation and de-esterification processes provides antitumoral activity [19]. The anti-tumorigenic activity of modified citrus pectin is generally related with its galectin-3 antagonism which is a member of lectin family and plays an important role in cancer and development of fibrotic disease [20]. Cancer stem cells are an important subgroup of cancer cells in tumor tissues. They exhibit increased resistance to drug molecules and other cancer treatment strategies. Their chemoresistance is mainly because of high expression of multi drug resistance proteins such as ABC transporters [21-23]. In addition, increased expression of galectin-3, is related to cancer development and metastasis, in tumor cells [24, 25] and cancer stem cells are shown in the studies [26]. There are some therapeutic strategies for inhibition of cancer stem cells including monoclonal antibodies and micro RNA technologies [23] and different types of pectin molecules may help the inhibition of cellular activity.

For tissue engineering applications pectin, is generally used in combination with other materials such as chitosan, hydroxyapatite, poly-L-lactone, and gelatin to increase its mechanical properties in the fabrication of 3D hydrogels [27, 28]. Pectin containing injectable biomaterials [29] and 3D matrices which supports cell attachment and proliferation can also be prepared by electrospinning process [30, 31]. In a study, vascular cell differentiation of mesenchymal stem cells (MSCs) was investigated on electrospun pectin hydrogel nanofibers. The cells were able to retain their viability for 14 days on the hydrogel nanofibers and their differentiation was changed with the stiffness of the hydrogels and was triggered in the direction of vascular cells [32].

Human body possesses limited regenerative capacity in organ or tissue repair, e.g. in pathologic conditions or in degenerative diseases. Regenerative medicine field highlights the function of stem cells for tissue repair and regeneration of organs because of their unique properties such as self-renewal, proliferation and differentiation into desired and various cell types [33, 34]. Mesenchymal stem cells are found almost in every tissue in our body such as, dental pulp, craniofacial bone, dental follicle, tooth germ, apical papilla, oral mucosa, gingiva, bone marrow, adipose tissue and periosteum [35-38]. The effects of pectin biomacromolecule on behavior of MSCs have been investigated. In a study, human amniotic membrane MSCs were cultured in hydroxyapatite/LM pectin composite gel structure. It was shown that MSCs preserved their osteogenic differentiation capacity and inhibition of T cell proliferation in the presence of LM pectin, also they did not trigger immune response [39]. Pectin/chitosan membrane can provide attachment, adhesion and proliferation of adipose-derived stem cells (ADSCs) [40]. Moreover, bone shaft

MSCs on chitosan/gelatin/pectin network (CGP) films exhibit high ALP activity, osteogenic gene expression and increased mineral formation capacity due to the suitable physicochemical and mechanical features of CGP network films. These results indicate that the network film has the potential to control the MSCs fate [28]. In another study, the biocompatibility of vancomycin hydrochloride including poly (L-lactide) fibers loaded silk fibroin/oxidized pectin hydrogels was shown by MTT assay. Human adipose tissue derived MSCs preserved their viability for eight day cell culture study [41].

Dental pulp stem cells (DSCs) are post-natal stem cell group which have similar properties to MSC with their self-renewal capacity, high proliferation rate and multilineage differentiation potential [42]. Dental pulp SCs can be isolated from third molars and dedicious teeth (DPSCs) without any invasive surgical process, so that they are a promising group of SC with their ease of obtain. Therefore, DPSCs are valuable and crucial MSC sources for tissue engineering and regenerative medicine studies [43]. Dental pulp stem cells involve neural crest stem cells and they have potential to differentiate into neurogenic, adipogenic, chondrogenic, osteogenic cell types [44, 45]. The studies which include pectin biomolecule and MSC interaction is generally based on bioscaffolds. Pectin as a part of biomaterial or as a soluble agent may trigger different signaling pathways in MSCs. To the best of our knowledge, there is no study in which the effects of the presence of soluble pectin in cell culture medium on human mesenchymal stem cells were investigated. In this study, it was aimed to investigate the effects of dissolved citrus pectin in culture medium on human dental pulp stem cells viability and morphology.

2. MATERIALS AND METHODS

Human Dental Pulp Stem Cells Culture

Human dental pulp stem cells (hDPSCs) which were isolated and characterized in a previous study [39] were a kind gift from Prof. Dr. Menemşe Gümüşderelioğlu, from Hacettepe University, Turkey. The cells were cultured in 15% (v/v) fetal bovine serum (FBS, Biowest, France) including alpha-Minimum Essential Medium (α-MEM, Biochrom, Germany). The antibiotics were used as [10 units/mL penicillin and 10 µg/mL streptomycin (Sigma, Germany), 50 µg/mL gentamicin (Sigma, Germany) and 0.25 µg/ mL Amphotericin B (Sigma, Germany)] at 37 °C in 5% CO2 atmosphere (Heraus, Germany). The cells were detached from the surface by trypsinization at 80% confluency and cryopreserved for further studies. Human DPSCs at passage 5 and 6 were used in the studies.

Pectin Solution Preparation

Stock pectin (Galacturonic acid ≥74.0%, Sigma Aldrich) solution was first prepared by dissolving 50 mg citrus pectin in 250 µl absolute ethanol [7]. After complete dissolution the mixture was added into 4750 µl α-MEM medium and was filtered through 0.22 µm syringe filter. After filtration, corresponding reagents were added to this solution to obtain 15% FBS (v/v), 1% (v/v) L-glutamin and 1% (v/v) antibiotic mixture in the final stock solution with 8.5 mg/mL pectin concentration.

Cell Viability Assays: Resazurin Application and MTT Assay

Human DPSCs were cultured on 24 well plates as $10x10^3$ cells/cm^2 in culture medium before application of pectin solution. After 24 hours, the culture media of the cells were changed with the fresh culture medium for cell control group, with pectin including media (0.43, 0.85 and 1.7 mg/mL) for sample groups and ethanol including medium (0.85%, v/v) for negative control group. Pectin concentrations were selected within the range of investigated concentrations according to the literature [7, 46, 47]. The cells were incubated in these media for eight days at 37 °C in 5% CO_2 atmosphere.

Resazurin stock solution (Sigma, Germany) was prepared as 0.8 mM in Ca+2 Mg+2 containing PBS (Biowest, France, PBS+). The solution was filtered through 0.22 µm syringe filter and kept at +4o C during

experiments. This stock solution was diluted by culture medium just before the application to the cells. The used medium on the cells were aspirated and fresh resazurin working solution (1/10 dilution in culture medium) was applied to the wells. The color change of the medium was observed after 4 hours at days one and eight. Due to the non-toxicity of resazurin on the cells, the used resazurin containing medium was collected for analysis after application. Cells were washed with PBS+ and fresh positive-negative control and pectin including media were applied to the cells. The cells were examined under inverted microscope (Leica, DM IL Led Fluo, Germany).

MTT analysis was carried out at the end of the culture period, at day 8. MTT working solution was prepared by 10 times dilution of 2.5 mg/mL MTT stock solution (Biofroxx, Germany) with culture medium without serum. Used culture media were aspirated from the wells and 600 µL MTT working solutions were applied to the cells. The cells were incubated with MTT working solution for 4 hours at 37 °C in 5% CO2 atmosphere. At the end of incubation, used MTT solutions were withdrawn and 0.04 M HCl containing isopropanol solution was applied to the wells to dissolve newly formed formazan crystals which are indication of mitochondrial activity of viable cells. The corresponding MTT absorbance values were recorded by UV-Vis Spectrophotometer (Agilent Cary 60) at 570 nm in comparison with 690 nm reference wavelength. Cell viability (%) was expressed in relation to the absorbance value of solvent control cells.

Live/Dead Cell Imaging

To assess the live/dead hDPSCs after pectin solution application, Ethidium Homodimer-1 (0.01 M, Eth-1, Abcam, UK) and Calcein AM (0.01 M, Sigma, Germany) dyes were used. Each dye was added into 1% L-glutamin containing PBS+ in 1/1000 dilution to obtain live/dead cell staining solution. Cells were washed once with PBS+ and live/dead cell staining solution was applied for 30 min at room temperature in dark. At the end of incubation period, staining solution was aspirated and the cells were washed once with PBS+ and visualized in PBS+ under a fluorescence microscope (Leica, DM IL Led Fluo, Germany) immediately.

F-actin/Nucleus staining

To observe the cellular cytoskeleton and cell nucleus, F-actin/nucleus staining was carried out. The cells were washed with PBS once and fixed in 4% formaldehyde (v/v in water) solution for 20 minutes at room temperature. After removal of the fixation solution, the cells were washed with PBS and 0.1% Triton X-100 solution in PBS was applied for 8 min to ensure cell membrane permeability. Dye solution was prepared in PBS containing 1% BSA (Bovine Serum Albumin, PBSA) with 2 µL/mL Phalloidin-iFluor 488 Reagent (Anti-Factin antibody, Abcam, UK) and 1 µg/mL DAPI (Sigma, Germany). After membrane permeability step, the cells were washed with PBSA and they were incubated in dye solution for 90 minutes at room temperature in dark. The cells were washed with PBSA for two times to remove unbound reagents and glycerol solution was added on the cells. The images of immunostained cells were taken under fluorescent microscope

Statistical Analysis

Statistical analysis was performed by one-way ANOVA and Post-hoc Tukey test by GraphPad Prism 8 statistic software program (GraphPad Software, USA).

3. RESULTS AND DISCUSSION

In this study, the effects of dissolved citrus pectin biomacromolecule on human dental pulp stem cells were investigated by means of cell viability and morphological analyses.

Resazurin sodium salt or resazurin including dye application is widely used for animal, yeast, bacteria and plant cells [48]. It is a non-toxic dye and the cells do not die after application; thus, it allows to track the viability of the same cell group during cell culture studies. The viability of MSCs also has been followed by resazurin dye in our previous studies [49]. In this study, the viability of hDPSCs was investigated by the application of 80 μM resazurin sodium salt solution. Mitochondrial activity in both control groups (ethanol group as a solvent control group and cell control group without any reagent application) and pectin applied groups were observed by the color change of culture media from light blue to purple and pink. There was no color difference between the groups on days one and eight according to our visual observations (data not given). Another assay which was carried out for the quantification of viability results of the same cell groups was MTT assay (Fig. 1). It was applied on day 8 and the results given in Fig. 1 were calculated by normalizing the corresponding absorbance values with respect to the absorbance values of solvent control group. It was clearly seen that, dissolved pectin application significantly decreased hDSCs viability when compared to the viabilities of control groups. The most decreased viability was observed in 1.7 mg/mL pectin applied group when compared to the control groups. In a study, the effect of citrus pectin and heat modified citrus pectin (3 mg/mL) in cell culture medium on HepG2 (liver) and A549 (lung) human cancer cell lines was investigated. It was concluded that both pectin types decreased cellular viability and some caspase independent cell death signals were observed in A549 cells [50]. In another study, viability of HaCaT (human keratinocyte) cell line in the presence of high molecular weight citrus pectin and modified citrus pectin with low molecular weight and low degree of esterification was investigated. It was stated that both type of pectin decreased cell viability in the 1-750 μg/mL concentration range [51].

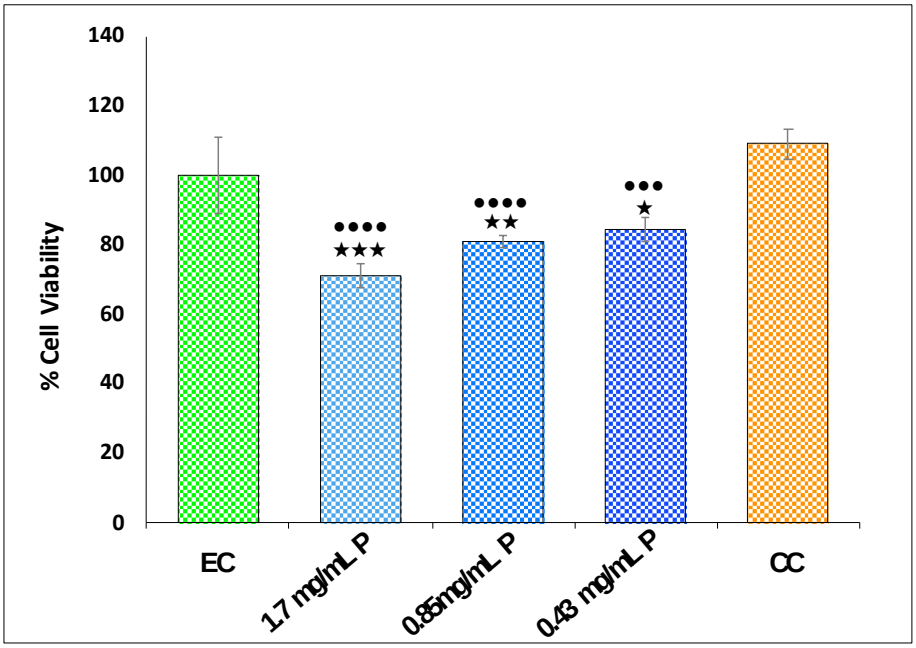

Figure 1. Viability of hDPSCs (%) by MTT assay on day eight. The absorbance values were normalized with respect to the absorbance values of ethanol applied group. P: Pectin, EC: Ethanol control, CC: Cell control "★" represents the significant difference in comparison with EC and "●" represents the significant difference in comparison with CC ($p<0.05$ ★; $p<0.01$ ★★; $p<0.001$ ★★★, ●●●; $p<0.0001$ ●●●●).

Inverted phase contrast images of hDPSCs were given in Fig 2. The morphologies of the cells in every group were as expected from MSCs with thin, bipolar, fibroblastic features (on day 1). After 8-day cell culture, the proliferation of the cells was significantly high, and it was not possible to analyze single cell morphology. However, their high proliferative behavior and growing capacity in multilayers were clearly observed in every group. As a result, there was not any significant change in the cell morphology which

can be related to decreasing cellular activity. Calcein-AM and Ethidium homodimer-1 staining allows visualization of live and death cells at the same time in live cell culture (Fig. 3). Non-fluorescent calcein-AM is a healthy cell membrane permeable die and turns into fluorescent calcein after intracellular esterase activity. Eth-1 is a nucleic acid binding reagent which allows visualization of cells with membrane damage (necrotic cells) shown in red color. Most of the cells were alive staining in green color and there were a few red points indicating death cells in the culture. Hence, decreasing mitochondrial activity may be a result of suppressed proliferation instead of activated necrotic cell death.

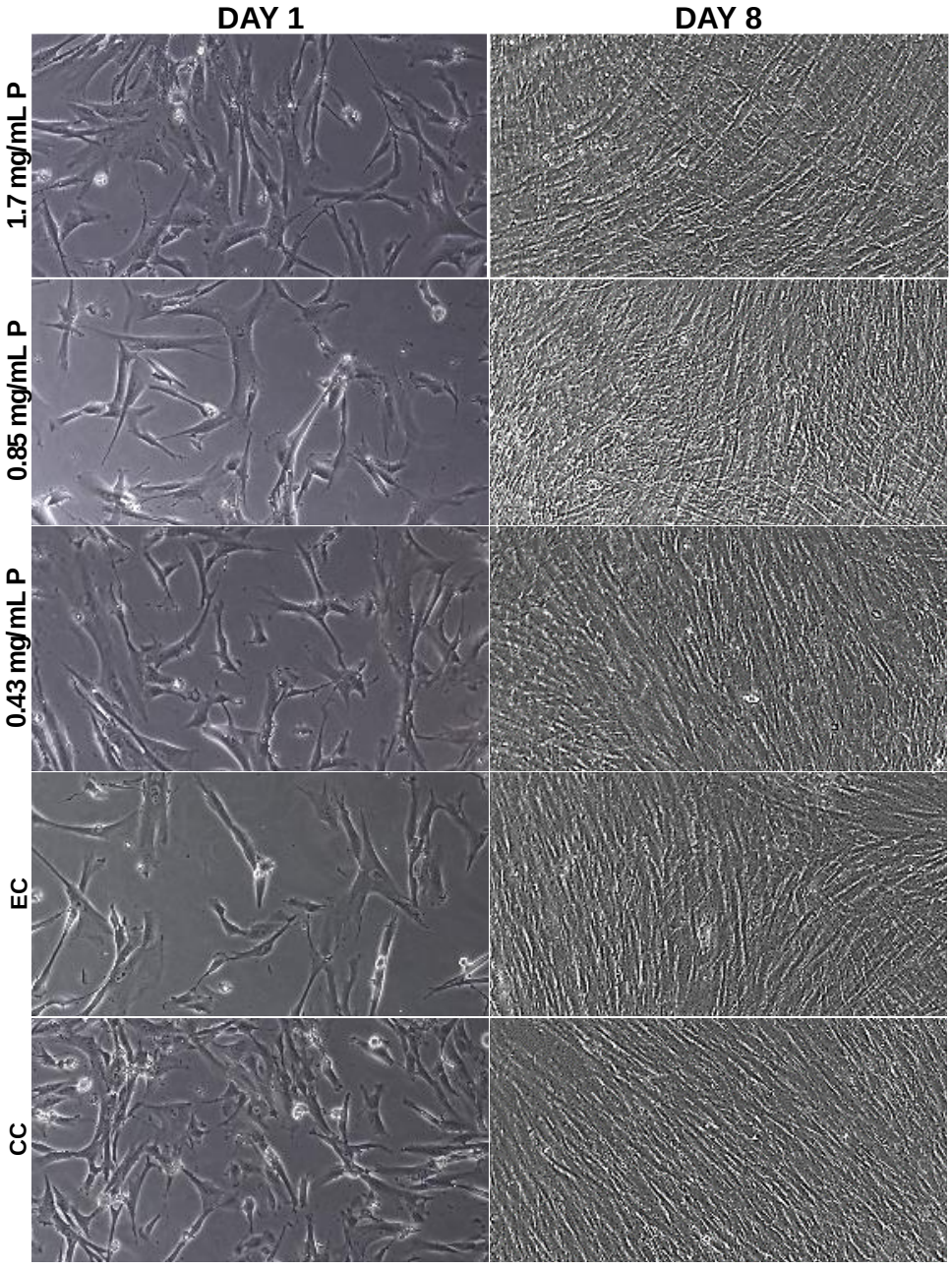

Figure 2. Phase contrast images (20X) of hDPSCs on days 1 and 8 before resazurin application under inverted phase contrast microscope. P: Pectin, EC: Ethanol control, CC: Cell control.

Mesenchymal stem cells spread on the surface and organize their filamentous actin stress fibers. In this study, F-actin/nucleus staining showed that hDPSCs fibrous actin filaments were organized in all groups, however, the difference in the morphology of the nuclei of certain cells in the pectin applied groups

was shown by arrows in Fig. 3. Condensed and crescent nuclei was shown in the cells which can be a sign of early stages of apoptotic cell death [52]. Induction of apoptotic pathways in cancer cells in the presence of different types of pectin has been discussed in many studies [53-55]. In a study, autophagy was also observed in A549 cells after heat-modified citrus pectin application in culture medium [50]. Hence, the crescent nuclear shape in hDPSCs of this study may be a sign of apoptotic cell death induced by the presence of pectin in culture medium which can be further validated by apoptotic markers and molecular studies. The effects of the presence of pectin in the 0.1 – 10 mg/mL concentration range were investigated in cancer cells related research in the literature [7, 46, 47]. In this study, 0.43 - 1.7 mg/mL pectin concentration range was selected in order to be comparable with the results in literature studies. However, higher pectin concentrations, longer cell culture periods and different types of pectin molecules should be investigated to observe more significant and specific changes in the viability, morphology and molecular signaling pathways of hDPSCs.

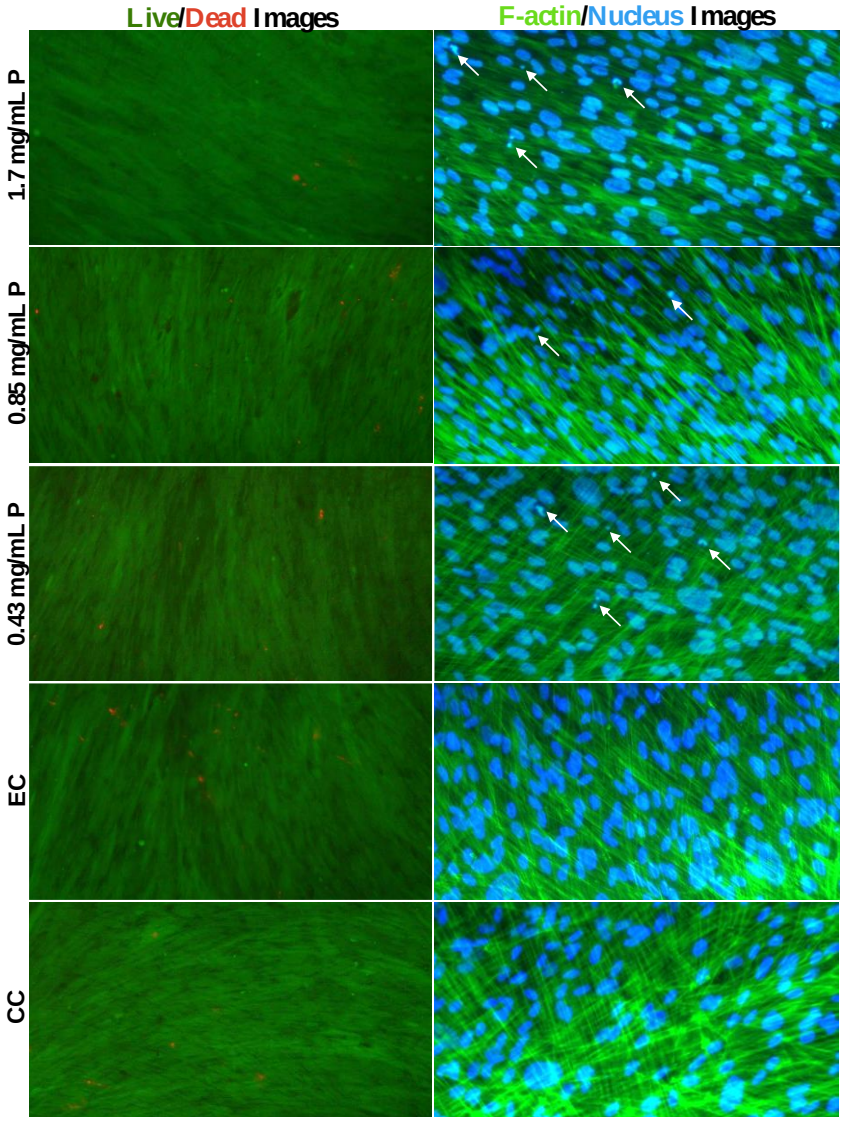

Figure 3. Fluorescence images of hDPSc on day eight. Live/dead cell analysis was performed at the end of culture using Calcein-AM for living cells and Ethidium homodimer-1 (Eth-1) for dead cells (10X). Calcein-AM incorporated living cells stained green color and Eth-1 permeable dead cells stained in red color. F-actin and nucleus staining was performed after fixation to analyze cell morphology (20X). Green color shows iFluor 488 conjugated anti F-actin antibody stained filamentous actin fibers, while blue color shows DAPI stained cell nucleus. White arrows show condensed and crescent cell nuclei. P: Pectin, EC: Ethanol control, CC: Cell control.

In this study, the decrease in the viability of hDPSCs may be explained by the interaction of pectin and galectin-3. Galectin-3 as a sub-group of carbohydrate binding galectin family is responsible for cell adhesion, proliferation, survival, tumor cell proliferation and metastasis [25]. It is also expressed in MSCs and can affect immunomodulatory properties of these cells [56]. The inhibition of galectin-3 related signaling pathways such as cell adhesion, survival and proliferation in the presence of different types of pectin were shown in the studies [53, 57]. Galectin-3 binding activity of pH modified citrus pectin was also clearly shown by enzyme linked immunosorbent assay [53] and suitability of some types of assays were studied [58]. Galectin-3 expression in cancer stem cells was also shown in a study [26] but it is a new research area and there are many points waiting to be clarified on this subject. Investigation of the interactions between cancer stem cells, galectin-3 and different types of pectin molecules will contribute to the development of alternative treatment strategies for cancer disease.

4. CONCLUSIONS

In this study, the effects of citrus pectin on human dental pulp stem cell viability and morphology were investigated. It was observed that proliferation of hDPSCs significantly decreased due to the presence of pectin in the cell culture medium for the selected concentration range. In addition, the shape of the nuclei of pectin applied cells showed crescent morphology when compared to the ellipsoid and smooth shape of nuclei in the control cells. This crescent morphology of nucleus may be a sign of apoptotic cell death induction by the presence of citrus pectin. It can be concluded that pectin biomacromolecule can significantly affect the viability and morphology of hDPSCs. Based on this information, it can be suggested that the evaluation of the effects of the pectin molecule on cancer stem cells may also be important.

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The Effect of Different Workout Programs on the Expression of the Genes Related to Oxidative Stress and Immune System

Research Article

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Abstract

Background: exercise conditions can affect the expression of genes. Our study investigated the effects of acute and chronic exercise on the expression of genes related to oxidative stress (Tfam, UCP2, UCP3) and immune system (IL-1α, IL-1β, IL-2, IL-8, IL12A, IL12B, TLR2, TLR4).
Methods: was formed with 24 people: 12 healthy females and 12 healthy males. Maximal oxygen use capacities of the participants were determined by the Bruce test protocol at the beginning and end of the 8-week training program. After calculating their maximal oxygen use capacity, each participant was given an acute running exercise on the tread mill at the speed and incline that the participant would reach to his/her maxVO2 until exhausted. The same people was built to continuous runs (50-70 %) for 8 weeks in a week, and two days of intermediate interval training program (80% and above). Venous blood samples were taken before and after acute exercise with immediately after chronic exercises. RNA isolation was performed using TRIzol Reagent from peripheral blood mononuclear cells. Gene expression was determined by Biomark Real-Time PCR (RT-PCR). Comparisons were performed by using two independent sample t-test and Mann-Whitney U for quantitative data and calculated gene expression values. The statistical significance level was taken as $p < 0,05$. Results: we found that acute exercise in women changed Tfam gene expression ($p < 0,05$). Chronic exercise changes the expression level of more genes (Tfam, IL-1β, TLR4) in women ($p<0.05$, $p<0.001$). There was only difference in Tfam gene in males ($p < 0,05$). Conclusions: changes in gene expression differed by sex in exercise. Our results indicate that different workout programs for females and males cause genes to work and they have a positive effect on their expressions and thus increase the efficiency of the exercise.
Keywords: Exercise, Gene Expression, Immune System, Oxidative Stress

1. INTRODUCTION

The effects of exercise on the immune system have begun to be examined especially in recent years and many studies have been carried out on this subject (Ntanasis, 2013, Meier, 2013, Bangi, 2000). While the results from these studies do not yet reveal the exact clinical relevance, long-term intensive exercises increase susceptibility to infections. Clinically, upper respiratory tract infections are usually reported after exhausting exercise (Eynon, 2009, De Nadal, 2011). Because of their basic role in the immune system, cytokines are one of the important research topics in physical exercise. A significant proportion of the cytokines secreted from the immune system are interleukins and their primary task is to stimulate the immune system cells. They are released temporarily in various immunologic, infectious and inflammatory diseases and it is indicated that plasma levels of cytokines such as IL-6, IL-1β, and TNF-alpha are increased especially in exhausting acute exercises (Flynn, 2003). Studies have shown that approximately 2 hours of daily regular moderate exercise reduces upper respiratory tract infection at compared to sedentary lifestyle, but this risk increases with intensive ultra exercises (LuzScheffer, 2019). Toll-like receptor (TLR), which is one of the key molecules in the immune system's defense against microbial infections, is an important contributor to the development of adaptation in the immune response to the monocyte, macrophage and dendritic cells. The activation of TLR receptor stimulates both the production of inflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-α, and the innate immune system. Studies report that TLR receptor increases in acute and chronic endurance exercise (Kumar, 2009, Lancaster, 2005). It has been reported that reactive oxygen species increase with the production of some immunity cell functions during the exercise. During long-term/intensive exercise, the amount of oxygen consumed changes depending on the intensity and type of the exercise, but in general, it causes the emergence of the free radicals (reactive oxygen species, ROS) that cause oxidative stress by increasing considerably compared to relaxation. In addition, ROSs plays an important role in regulation of cell signaling and gene expression (Kocakulak, 2020, Kocakulak, 2019). Physical exercise is seen as the reason of the changes in the skeleton muscle genes expression of a many people. However, it is not known exactly which mechanism regulates gene expression of important genes involved in metabolic stress or metabolic adaptation (Gleeson, 2007, Borras, 2003). The purpose of this study is to investigate the effect of the acute and chronic exercises on the possible changes that can occur in the expressions of 7 genes selected as being related to oxidative stress (Tfam, UCP2, UCP3) and immune system (IL-1β, IL12B, TLR2, TLR4). Thus it has been tried to explain if the increases and decreases in the expression levels of these genes are related to exercises.

2. METHODS

Study Group: The research was conducted with total 24 people, 12 of whom were healthy females and 12 healthy males. Subjects who do not exercise regularly, do not smoke, do not take any additional nutrients are included in the study. Our study protocol was approved by Erciyes University Ethical Committee and the study was conducted in accordance with Helsinki Declaration and local law (No:2012/68). Maximal Aerobic Capacity (V̇o2 max): The maximal oxygen-using capacities were determined proportionally by applying the Bruce Test Protocol (Bruce, 1949). The first blood sample was taken to measure the resting values of the volunteers after calculating the amount of oxygen consumed (V̇o2 max) and 2 days later. After 10 minutes of warm-up and stretching exercise, maximal exercise running test was applied. Maximal exercise running test: According to the Bruce Test Protocol, each participant did the running exercise until it was exhausted, at the speed and slope it reached to V̇o2 max. Exercise participants It was completed by taking its own declarations and looking at the target heart rate (220-age). Blood samples were taken after exercise to determine the effect of acute exercise. Then 8 weeks of continuous runs and medium interval training started. Training program: The participants participated in the training program 3 days (Monday, Wednesday, Friday) a week for 8 weeks. Continuous Running Training Method: The participants were given a running exercise 1 day per week for 8 weeks between 25-60 min and 50-70% of

the target heart rate. İntermediate interval training program: The participants were given intermediate interval training program 2 days a week, for 8 weeks. The heaviness of the training was determined depending on the (80% and above) target heart rates of the volunteers. The scope of the training was determined as 4800 meters. The amount of oxygen consumed after the training was finished was recalculated. After 2 days, maximal exercise running test was applied and 10 ml venous blood samples in tubes having EDTA were taken exercise after to see the effect of chronic exercise. The heights, weights, systolic and diastolic blood pressures and heart rates of the participants was recorded before and after from maximal exercise running test.

RNA Isolation and gene expression studies: Genetic studies were carried out at Erciyes University Genome and Stem Cell Center. RNA was isolated from 2ml blood samples taken from the groups (TRIzol, Roche, Germany) (Nilsson, 2008, Bayram, 2016). The quality and quantity of RNA was measured by BioSpec-Nano Spectrometer. Complementary DNA (cDNA) was obtained from RNA by RT2 HT First Strand (Qiagen) kit. While complementary DNA was being synthesized, it was left 42°C 15 min and 95°C 5 min incubation. Gene expressions were determined by Biomark Real-Time PCR (RT-PCR). Biomark Real-Time PCR (Qiagen) was used for expression study. While expression study was being held, it was incubated at 95°C 10 min and throughout 40 cycles 95°C 15 sec, 60°C 60 sec. Each sample was worked on twice and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was selected as the housekeeping gene. Delta delta Ct method (2-ΔΔCT) was used for the relative quantification of the samples that were normalized by Glyceraldehyde-3-phosphate dehydrogenase (Jemiolo, 2004, Catoire, 2012).

Statistical Analysis: Data was collected with Biomark Real Time PCR analysis software using linear baseline correction method and auto global Cq threshold method. First, each gene was extracted from each gene expression control gene expression. These values are kept as ΔCT. The maximum ΔCT value of that gene was then subtracted from each sample ΔCT value for each gene. ΔΔCT values were obtained at this point. System given Cq values of 999 and values larger than 23 haploid genome equivalents (HGEs) was considered as unreliable and removed. GeNORM was used to evaluate the expression stability of genes, and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize the RT-PCR data as an internal control. Data normalization was performed by using the 2-ΔΔCT method. For all 8 groups, all binary comparisons were performed using the Mann-Whitney U test with calculated gene expression values. Statistical significance level was taken as $p<0,05$.

3. RESULTS

The average age of the participants was 21.88 ± 2.44 years for females and 23.8 ± 4.1 years for males, and the average height was 162.13 ± 5.83 cm for females and 174.7 ± 6.9 cm for males (Table 2).

Table.2. Some physical and physiological characteristics of the participants

Variables	Groups	Bruce test BE Avg±SD	2.Bruce test AE Avg±SD	t	P
Weight (kg)	Female (n=12)	58.60 ± 2.04	56.24±1.65	4.2	0.001**
	Male (n=12)	72.1 ± 9.7	71.3± 9.5	1.6	0.116
BMI (kg/m^2)	Female (n=12)	22.29 ± 2.43	21.37±1.98	4.5	<0.001**
	Male (n=12)	23.5 ± 2.5	23.3±2.3	1.6	0.117
Heart Rate (rate/min)	Female (n=12)	86.70 ± 6.7	98.90±7.98	5.3	0.001**
	Male (n=12)	81.60 ± 8.4	98.20±6.32	5.1	0.001**
Max VO2	Female (n=12)	35.74±2.5	46.16±3.25	5.1	0.001**
	Male (n=12)	51.8 ± 4.7	56.5 ± 3.3	4.8	0.001**

Paired Samples T Test / SD.: Standarddeviation / *p<0.05 **p<0.001

B.E.=Before Exercise A.E.=After Exercise BMI= Body Mass Index

According to the statistical analysis results, it was found that heart rates increased significantly in both females and males after exercise (p<0.001). After exercise, body weights (p<0.001) and body mass index (p<0.000) decreased significantly in females. No difference was found in males (p>0,05). The changes in the expression of 7 genes related to oxidative stress (Tfam, UCP2, UCP3), immune system (IL12B, IL-1β, TLR2, TLR4) are shown in the following tables.

Table 3. Change in gene expression before and after acute maximal exercise protocol in women and men.

Gene	Groups	**BE- F** Median - **IQR**	**AE- F** Median - **IQR**	P-value*
Tfam	Female	5.48 ± 2.387	6.683 ± 1.48	**0,020***
	Male	7.909 ± 1.22	7.846 ± 0.593	0,713

Mann Whitney U test / IQR: Interquartile range *p<0.05 B.E.=Before Exercise A.E.=After Exercise

There was an increase in *Tfam* gene expression after exercise in women. (p<0.05, Table.3).

Table 4. Change of gene expression before and after 8 weeks training program in both women and men.

Gene	**Groups**	**BE- F** Median - **IQR**	**AE - F** Median - **IQR**	P-value*
Tfam	Female	5.48 ± 2.387	7.773 ± 0.216	**0,000****
	Male	6.298 ± 2.373	7.887 ± 0.363	**0,039***
IL-1β	Female	14.993 ± 3.2	1.997 ± 11.629	**0,001****
	Male	11.283 ± 3.321	0.5 ± 12.345	0,228
TLR4	Female	2.863 ± 0.38	3.364 ± 0.332	**0,019***
	Male	3.598 ± 0.448	3.834 ± 0.283	0,151

Mann Whitney U test / IQR: Interquartile range *p<0.05 **p<0.001 B.E.=Before Exercise A.E.=After Exercise

There was a significant change in Tfam, IL-1 β, TLR4 gene expression after 8-week training program compared to pre-exercise in females. There was only one gene difference in males. (*p<0.05 Table 4).

4. DISCUSSION

Exercises have both positive and negative effect on immune system. After extended heavy exercises, minor diseases especially such as sore throat and flu are more frequent in athletes compared to the general population. Due to the high incidence of increasing of the breathing depth, exposure to the pathogens in the air also increases (Kocakulak, 2020). In our study, it is seen that Tfam gene expression of both females and males that we had determined as related to oxidative stress changed especially in the entire exercise protocols. While it increased in women after acute and chronic exercise, it increased in men after chronic exercise. The change after acute exercise is not significant. It was found that Tfam gene expression was affected for both females and males in the exercises with increased intensity. Oxidative stress and antioxidant capacity has been compared between females and males in a lot of studies up to today. There is no study among these studies that has reported difference between genders related to the oxidative stress level (Kocakulak, 2019, Gleeson, 2007). It has been emphasized that the differences are due to handling sample groups such as trained and untrained people and elderly population. Innate defense system of the body keeps the free radicals under control against the harms of the reactive oxygen species (Borras, 2003). Normally there is a delicate balance between reactive oxygen species and antioxidant activity. Exercise can increase the formation of free radical by accelerating metabolic processes. In a severe exercise, the use of oxygen in skeletal muscles can increase 100-200 times (Ahmetova, 2010). Superoxide radicals normally occur 1-2% in the mitochondrial respiratory chain. It has been report that this radical has occurred in the ubiquinone-cytochrome step being related to ubisemiquitinone oxidation (Parise et al., 2005). It is stated that the TNF-α and IL-1b levels increased twice and IL-6 level increased up to 100 times after a marathon race. According to studies the duration and intensity of the exercise affects the cytokine profile.

(Moldoveanu et al., 2000) performed a 20-minute cycling ergometer, 20-minute treadmill and again cycling ergometer test by dividing a 3-hour exercise into three. They took blood samples just after the exercise, 2 hours later and 24 hours later and they investigated TNF-α, IL6, IL-1β gene expressions in the mononuclear cell in blood and the plasma levels. It was reported that TNF-α, IL6, IL-1β plasma concentrations increased significantly just after the exercise, they continued to increase 2 hours later, and they started to return normal levels but there was no change in gene expressions. Although there are a few studies on TLR4 gene, its expression levels were investigated in both acute aerobic exercise and chronic endurance exercise, and a decrease was determined in the monocyte cell surface expression of the Toll-like receptor (Flynn, 2003) had old females have low endurance training as being 10 repetitions, 3 series and 9 trainings at 80% of 1 RM 3 days a week for 10 weeks, and TLR4 was found lower compared to the sedentary control group. Oliveira and Gleeson had young and healthy males (VO2 Max 75%) have total 1,5 h highly intensive interval cycling exercise 3 times a week for 2 weeks and they investigated the expression levels of TLR4 and TLR2. According to this study, TLR4 gene expression decreased in the 0 and 1st hour compared to pre-exercise, it returned to its previous level 4 hours later, and there was no change in TLR2 gene expression. No difference could be found in some studies. These results support our study and it is considered that the different results are due to the sample groups handled and training designs (Olıveıra-Chıld, 2013). In studies it is reported that immune system is not affected by intensive and long-term exercise and these result can change depending on the ages of the participants and training management (Radom-Aizik, 2010). It is thought that the differences between females and males occurred in our study are resulted from the intensity and duration of the exercise, individual differences, demand for oxygen, the activity levels of the individuals, energy needed during the exercise, and oxidation, which is the main principle of energy consumption. When the oxygen consumption of the participant was considered, it was determined that after the exercise it increased considerably in females and males when compared to before the exercise. Depending on the severity and intensity of the training, oxygen use capacity (V̇o2 max) develops. It is known that heavy trainings affect oxygen use capacity negatively. It is seen that the change in the expressions of the genes that are determined related to immune system and oxidative stress have not affected

the oxygen use capacities of the participants negatively. Not all genes found in humans can be actively present at the same time. Gene expression is affected by several factors.

Determining gene expression or transcription level always provides a basis for gene function studies. The data thus obtained, the number of genes studied, the number of samples included in the study in terms of the type of exercise and bears the distinction of being the first in Turkey to study this issue. According to our study, after acute exercise, 8-week interval exercises, differences occurred due to the gender and due to the characteristics of the genes that are determined related to the oxidative stress (Tfam, UCP2, UCP3) and immune system (IL-1β, IL12B, TLR2, TLR4). However, it is not known if the increases and decreases are resulted from different exercises, from the physiological characteristics of the volunteer groups participated in the study, from the states of nutrition and rest or from the levels of the daily activity. It is thought that additional studies are necessary to make the distinction in question. However, our present findings suggest that there are significant increases and decreases in gene expression especially in some genes before and after acute and chronic exercises. These results indicate that the selected genes are activated and work by exercise, and it is thought that genetic markers may be important since they play an important role in increasing the efficacy of the exercise on individuals.

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Bacteriological Assessment of Selected Hand-Pumped Boreholes Water Sources in Malete Environs, Kwara State Nigeria.

Research Article

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Abstract

A significant source of water for domestic use in Nigeria and developing countries is groundwater. Groundwater is generally very clean but pollution can result, as nutrients and toxic chemicals find their way into it. This study assessed the microbial quality of water (hand-pumped boreholes) at six areas in Malete environs. Water samples were collected in triplicates and subjected to physicochemical (temperature, pH, conductivity, turbidity, salinity and hardness), microbiological (heterotrophic and coliform counts) and molecular analysis using standard methods. The temperature generally ranged from 26.45±0.20 to 29.3±0.19 ºC, pH ranged between 7.6 ± 0.00 and 8.2 ± 0.012. Turbidity was generally <5 NTU, conductivity was <250µS/cm and hardness was <150 mg/L. The values were within acceptable range stated by World Health Organization (WHO) and the National Standard for Drinking Water Quality (NSDWQ). *Escherichia coli*, *Stapylococus aureus*, *Streptococcus pneumoniae* and *Klebsiella oxytoca* were found in some water samples. Faecal coliform was found in 83.3% of the hand-pumped boreholes, these high prevalence of contaminated water is attributable to the largely contaminated environments around some of the wells which was littered with various forms of refuse. Public awareness on the dangers associated with the construction of hand-pumped boreholes at a distance less than 15 meters away from the septic tank should be increased. Also hand-pumped boreholes water samples that do not meet the standard should be treated before consumption.

Keywords: Hand-pumped boreholes, faecal contamination, portability, anthropogenic activities

1. INTRODUCTION

Lack of potable water is a problem, which affects billions of people worldwide. About 1.1 billion people do not have access to any form of source of potable water and about 1.6 million deaths result annually as a direct consequence of lack of access to potable water and basic sanitation (WHO, 2016). Although water occupies a large portion (70%) of the earth's surface, availability of improved water source for

domestic use especially in Nigeria and other developing countries in the world is still very low. Potable water is one of the most considered necessities of all urban and rural amenities and is requisite for man's activities. Both natural influences and human activities are factors responsible for the quality of any body of surface or ground water (Kolawole et al., 2013). Regional government established Water Boards Corporation to provide safe water for the communal folks. Federal Government of Nigeria resulted in the establishment of some federal agencies which include River Basin Development Authorities, the Federal Ministry of Water Resources (1976) and National Water Resources Institute (1977) in early seventies as a result of drought to formulate policies, manpower training, research and give advice on domestic water supply to the communities.

However, just before the commencement of this programme, only 22% of the rural and 55% of the urban population could use portable water. The situation has increased only marginally. The delivery of water supply systems, especially to the Rural Areas in Nigeria, has generally been inadequate in relation to desired goals and objectives.

Only 50% of the urban population and about39% of rural communities have access to portable water. To solve problems of water shortage in the rural communities, World Bank and other related organizations postulated that hand pump well water should be provided to combat this shortage.
Sewage or human and animal waste are the major sources of danger accompanying with drinking water sources (Hague, 2019). Locating drinking water system (wells and boreholes) close to a refuse dumpsite or landfill is another problem. Therefore, regular and frequent examination of water is essential as contamination may be intermittent and may not be detected by simple tests.

Due to lack of awareness in Nigeria, groundwater contamination is one of the least recognized environmental problems since contamination are not as noticeable as those affecting surface water (Adeyemi et al., 2007).

Over the last eras access to safe potable water has improved in Nigeria and almost every part of the world, around 1.1 billion people are still facing water shortage and over 2.6 billion worldwide lack access to adequate hygiene resulting in water borne sicknesses such as cholera, diarrheal disease, botulism, *E. coli* infection, dysentery, legionellosis, leptospirosis, salmonellosis, typhoid fever and vibrio illness (Adogo et al., 2016).

Most borehole locations are very close to sanitary pipelines, which transverse the septic tank absorption fields. The sewer and the cast pipes for the transportation of water are subjected to leakages, and sometimes experience accidental backflow or back seepage of polluted water. This may occur from toilets and wash bowls, resulting in the contamination of water supply pipes through leakages. Thus, fecal coliform bacteria might be introduced into the well.

E. coli presence in water suggests presence of enteric fecal pollution (Ivey et al., 2006), this organism is the major causative agent of diarrhoea, urinary tract infection, hemorrhagic colitis and haemolyticuraemia syndrome.

This study therefore, was designed to assess the qualities of water samples from hand pump well, in addition taking into consideration the EPA'S recommendation for acceptable distance between a water well and a septic tank in different communities in Malete environs, Kwara State Nigeria.

2. MATERIALS AND METHODS

Study Area

This study was conducted in Moro Local Government Area (LGA) of Kwara State. The LGA has an area of 3,272 km² and an estimated population of 146,310 (Thomas, 2017). The LGA is made up of a conglomerate of agrarian villages. The physical environment of the primary sources of water in the villages, which were hand-pumped well were assessed. The sampling sites are (A: Akorede, B: Ashomu, C: Government secondary school Malate, D: Safari Village hostel, E: Isalebaale and F: Okete).

Sample Collection

Water samples were aseptically collected to prevent cross contamination in triplicates to pre-sterilized 250mL bottles from six different communities in Moro LGA. The sampling was done in August, 2019 which corresponds to the rainy season. The anthropogenic activity around the hand-pumped boreholes at each location were recorded (Aneja, 2003).

Sampling Analysis

Standard methods were used in carrying out the physicochemical and bacteriological analysis of the water samples.

Determination of Physiochemical Parameters

Determination of Temperature

Mercury bulb thermometer calibrated in ^{0}C was used to measure temperature of each sample collected on site while electrical conductivity was measured with a CDM 83 conductivity meter (Radio Meter A/S Copenhagen, Denmark).

Determination of pH and Other Parameters

The pH of the water samples was measured using the electrometric method with a pH meter (model: HP 2211 ph/ORP meter) on site while other parameters such as total dissolved solids (TDS), total suspended solid (TSS), total solid (TS), total alkalinity and bicarbonate were determined colorimetricaly by Spectronic 20 (Gallenkamp,UK) (APHA, 1981).

Bacteriological Analysis

Enumeration of Total Heterotrophic Bacteria Counts (THC)

Total viable count was done using the standard plate count method for the examination of water and waste water. Tenfold serial dilutions of 10^{-1}, 10^{-2}, 10^{-3},1mL of the 10^{-2} and 10^{-3} dilutions were used to seed the properly labelled plates. The sterile molten nutrient agar cooled to about 45^{o}C was poured into the appropriate plates and swirled to ensure proper mixing of the inoculum with the medium. The plates were then allowed to set and incubated at 37^{o}C for 24hrs. The plates were observed for growth after 24hrs and the numbers of discrete colonies on plates were observed and recorded in cfu/mL (Fawole and Oso, 2004).

Enumeration of Total Thermotolerant (faecal) Coliform Counts

This method of multiple tube test technique used for total coliform was also used to enumerate total thermotolerant coliforms. The only difference is that the temperature was increased from 37^{o}C to 44^{o}C for 24 to 48 hours. The high temperature at which the organisms grow is a characteristic of their uniqueness as indicator organism of faecal pollution of water (Fawole and Oso, 2004).

Isolation of Pure Cultures

Pure cultures were obtained by transferring different and distinct colonies into sterile solid nutrient agar plates using sterile inoculating loop and then streaked. The plates were then incubated and sub-cultured until satisfactorily pure cultures were obtained. The pure cultures obtained were then inoculated further onto agar slants in McCartney bottles, incubated at 37^{o}C and stored in the refrigerator at 4^{o}C. The stock cultures were to serve as a source of reference whenever tests would be carried out on the isolates.

Characterization and Identification of Bacterial Isolates

The characterization and identification of bacterial isolates were based on the colonial morphology and biochemical characteristics. The colonial morphology of the microorganisms were based on the shapes, size, optical character, consistency, elevation and pigmentation, while the cellular morphology was determined through microscopic examination and staining techniques. After characterization, tentative identification was done using Bergey's Manual of Determinative Bacteriology (Cheesbrough, 2006).

Molecular Characterization of Bacterial Isolates from Water Samples

16SrDNA sequences processing Genomic DNA of the isolate was extracted following standard method. PCR amplification was carried out as follows: A pair of universal primer of 27F (5-AGAGTTTGATCCTGGCTCAG-3) and 1492R (5- GGTTACCTTGTTACGACTT-3) was added to 2.5µl PCR buffer containing 2µl dNTP mix, 0.2µl Taq DNA polymerase,5µl3 of DNA template and 12.8µl sterile distilled water made up 25µL reaction volumes. The thermo-cycler PCR conditions were as follows: denaturation (92◦C/1min), annealing (54◦C/ 1 min) and extension (72◦C / 1 min) in 30 cycles (Miller et al., 2013). PCR product was loaded into well and electrophoresed on agarose gel at 120V for 45 minutes using a maxi gel system. The band on the gels was visualized by ultraviolet trans-illumination (Uvitec, Cambridge, UK), then sequenced. The 16S rRNA sequences of the isolate and similar sequences downloaded from NCBI database using the BLAST search program were aligned using the multiple sequence alignment generated using MEGA 6.0.

Statistical Analysis

Results of the Statistical analysis were expressed as mean and Standard deviation of triplicates and were statistically analyzed using ANOVA of SPSS statistical package of version 16.0. Values were considered significant at $p<0.05$

3. RESULTS

Anthropogenic Activities and State of Surrounding of the Wells

Plate 1 shows the sampling sites as well as anthropogenic activities around the boreholes that were sampled, while Table 1 shows the sanitary surveillance of the surroundings of the boreholes that were sampled.

The descriptions of the sampling sites are shown in Table 1. About 33.3% of the hand pump well are located close to the refuse sites while only 16.7% located close to septic tanks. The pH values ranged from 7.6 to 8.2, the temperature ranged between 26.45± 0.20ºC to 29.3 ± 0.19ºC, turbidity (0.97 to 2.30 NTU), dissolved oxygen (5.35 to 6.20mg/L), salinity (0.017 to 0.060 mg/L), conductivity (64.4 to 97.4 µS/cm) while the total hardness ranged between 3.27 and 5.00ppm (Table 2). The highest mean heterotrophic count was 2.93×10^{4}±6.67 cfu/mL for site D and the least was 1.06×10^{2}±3.33 cfu/mL for site A, 75.0±0.63 cfu/mL was the highest mean coliform count for site D and the least was 5.0±0.24 cfu/mL for site B, while the highest total faecal coliform count was 65 ±0.18 cfu/mL with no faecal coliform in site B (Table 3). The Agarose gel Electrophoresis of the amplified DNA of the four bacterial isolates is shown in Figure 1.

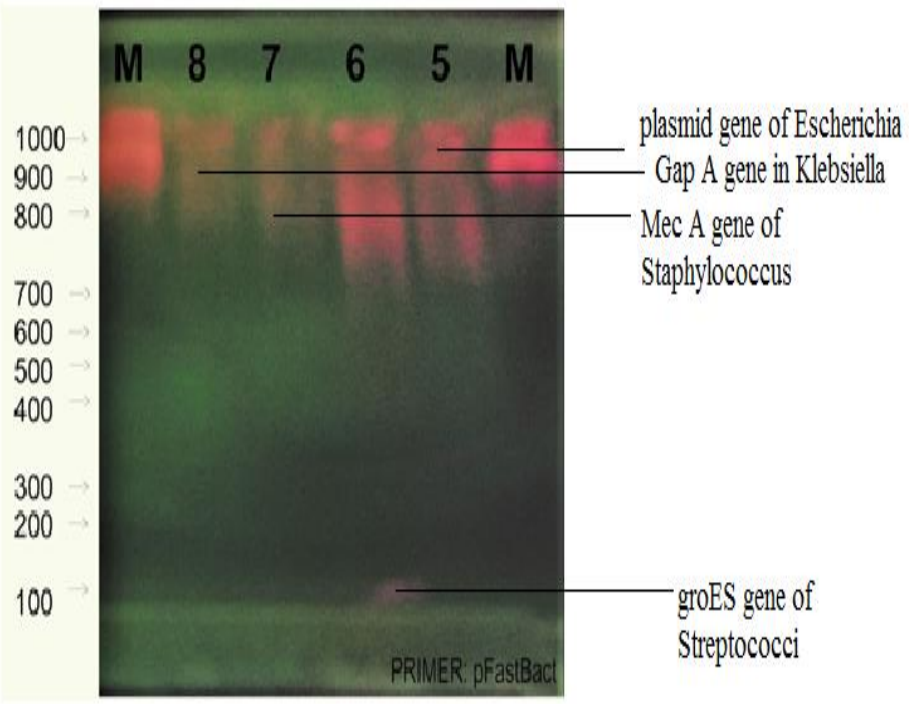

Figure 1. Gel electrophoresis of bacterial DNA of the isolates from hand pump well waters Key: M: DNA ladder.

Plate 1 (A-F): Surrounding of the sampling site showing anthropogenic activities Key: Site A: Akorede; Site B: Ashomu; Site C: Government Secondary School Malete; Site D: Safari Village hostel; Site E: Isalebaale and Site F: Okete.

Table 1: Sanitary Surveillance of the Sampling site

Sample site	Refuse/Solids dump	Animal dung	Floor covering	Stagnant water	Water Appearance	Proximity to drainage/ septic system
A	Close	Present	Fair	Present	Cloudy	Far
B	Close	Absent	Poor	Present	Clean	Far
C	Far	Present	Fair	Present	Fairly clean	Far
D	close	Absent	Good	Absent	Brownish and offensive	Very close
E	Far	Present	Poor	Absent	Clean	Very close
F	Close	Present	Poor	Present	Fairly clean	Far

Key: Site A: Akorede; Site B: Ashomu; Site C: Government Secondary School Malete ; Site D: Safari Village hostel; Site E: Isalebaale and Site F: Okete Far (11- 14 Meters), Close (6-10Meters) and Very close (0-5 Meters).

Table 2: Physicochemical analysis of hand pump well water samples

Parameters	**Site A**	**Site B**	**Site C**	**Site D**	**Site E**	**Site F**	**WHO/SON Standard**
Colour	Cloudy	Clean	Clean	Brownish	Coloured	Coloured	Colourless
Temperature (ºC)	26.5±0.24[a]	29.3±0.19[a]	28.60±0.26[a]	280.2±0.21[a]	27.50±0.23[a]	26.45±0.2[a]	Ambient
pH	7.86±0.01[a]	7.83±0.00[a]	7.80±0.00[a]	8.20±0.012[a]	7.76±0.03[a]	7.6±0.00[a]	6.5-8.5
Turbidity (NTU)	1.10±0.02[a]	1.20±0.00[a]	0.97±0.012[a]	2.30±0.02[b]	1.30±0.00[a]	1.1±0.01[a]	<5
Dissolved Oxygen (mg/L)	5.50±0.13[a]	5.46±0.21[a]	5.43±0.01[a]	6.20±0.00[b]	5.57±0.05[a]	5.37±0.10[a]	6.5-8
Salinity (mg/L)	0.06±0.00[a]	0.017±0.06[b]	0.037±0.02[c]	0.01±0.00[b]	0.043±0.00[c]	0.037±0.02[c]	<1000
Conductivity (µS/cm)	87.20±0.01[a]	97.40±0.01[c]	93.41±0.00[c]	83.15±0.02[a]	90.01±0.00[c]	64.4±0.00[b]	250
Hardness (mg/L)	3.57±0.12[a]	4.2±0.00[b]	3.76±0.02[a]	4.13±0.01	3.27±0.02[a]	5.00±0.00[c]	<150

Values represent means ± standard error of means. Values with same superscript have no inferential difference while those with different superscripts have inferential difference at 95% confidence interval. *(n=6) along rows.*

Table 3: Bacteriological Analysis of the collected water samples

Sample	Total Heterotrophic count (THC) (cfu/ml)	Total coliform count (TCC) (cfu/ml)	Total faecal count (TFC) (cfu/ml)
A	$1.06\times10^{2}\pm3.33^{a}$	12.0 ± 0.58^{c}	4.0 ± 0.57^{a}
B	$1.56\times10^{2}\pm6.67^{a}$	5.0 ± 0.24^{a}	0.00
C	$1.26\times10^{3}\pm6.67^{b}$	9.0 ± 0.55^{b}	6.0 ± 0.54^{a}
D	$2.93\times10^{4}\pm6.67^{c}$	75.0 ± 0.63^{f}	65.0 ± 0.18^{d}
E	$2.10\times10^{4}\pm5.77^{c}$	23.0 ± 0.12^{e}	48.0 ± 0.58^{c}
F	$1.16\times10^{3}\pm3.33^{b}$	17.0 ± 0.26^{d}	11.0 ± 0.65^{b}

Key: Site A: Akorede; Site B: Ashomu; Site C: Government Secondary School Malete; Site D: Safari Village hostel; Site E: Isalebaale and Site F: Okete *Values represent means ± standard error of means.* Values with same superscript have no inferential difference while those with different superscripts have inferential difference at 95% confidence interval. *(n=6) along column.*

4. DISCUSSION

Hand pumped well water and other underground water sources are major source of drinking water in Nigeria, especially among local populace, who either have no access to public pipe borne water supply or cannot afford to rely on treated bottle water for their consumption and domestic usage (Akpoveta et al., 2011). The environments surrounding the hand pumped well were largely contaminated and littered with various forms of refuse. This is large due to anthropogenic activities of the populace around pumped well. All the hand pumped wells water studied, were less than 15metres from the nearest septic tank, and the results revealed a high rate of contamination as all the hand pumped wells analyzed were contaminated with coliforms. Arwenyo et al. (2017) and Rohmah et al. (2018) who have investigated the effects of septic tank proximity to drinking water wells. They have reported similarly high values of total coliforms in different groundwater samples in their studies conducted within the country and outside the borders of Nigeria.

The temperature of the water samples in this study generally ranged from 26.45±0.2 to 29.3±0.19ºC. No substantial variation in the temperature of the water samples. Sabo *et al*. (2013), recorded temperatures of between 26.38ºC to 29.93ºC for hand pumped well water samples in Gombe state. Temperature accounts for a whole lot of other physicochemical parameters and it is also a major determinant of the type of microorganisms found in the water. The pH of water is generally accepted to be neutral (7.0) (UN, 2015). Results from this study revealed variations in the pH of the water samples from 7.6 to 8.2, and thus falls within the values of 6.5-8.5 recommended by the Environmental Protection Agency (UN, 2006). Results

from this study showed that turbidity across the hand pumped wells water samples were generally low. It ranged from 0.97 NTU to 2.3 NTU. The turbidity noted in this study was within the limit set by Nigerian standard for drinking water quality (UN, 2015). Similar results were obtained by Adogo *et al*. (2016)

No statistical difference was found among the different hand pumped well water samples studied at $p< 0.05$ in terms of pH, temperature, turbidity and dissolved oxygen. The range of dissolved oxygen obtained in this study showed that very little organic pollution occurred in the water body, and was well within the standards set by NSDWQ (NSDWQ, 2007). The results obtained in this study was relatively higher than the 1.3 mg/L and 1.8 mg/L reported for private and public boreholes respectively by Ukpong and Okon (2013). Salinity was generally low in this study. It varied from 0.01 mg/L to 0.06 mg/L. Similar results were obtained by Asuquo and Etim (2012) and Adogo *et al*. (2016). High level of salinity in borehole water samples are usually attributed to the presence of refuse dumps or septic tanks close to these underground water sources (Anne *et al*., 2015). Results from this research revealed that hardness of the hand pump well water samples ranged from 3.27 mg/L to 5.0 mg/L and were within recommended limits of <150 mg/L, it shows significant different at $p< 0.05$. These results are in agreement with the reports of Sabo *et al*. (2013) and Adogo *et al*. (2016).

Based on the morphological and biochemical characteristics, 4 major and distinct bacterial isolates *Klebsiella* sp., *Staphylococcus aureus, Escherichia coli*, and *Streptococcus* sp. were isolated. Both non-fecal and fecal coliform bacteria are the most contaminant organisms. The water samples with low bacteria and total coliform counts have better quality for domestic use than the ones with the highest counts of both bacteria and total coliform counts. Akinyemi et al. (2006) in their earlier studies in Lagos and Ibadan reported that water for drinking and domestic purposes from well and borehole were grossly contaminated with pathogenic organisms. Donderski and Wilki (2001) reported that fecal coliform bacteria number is an indication of the contamination size by fecal substance and also, that the total number of heterotrophic bacteria is a reflection of contamination extent by the easily decomposable organic matters.

In this study, coliforms were found in all the water samples, while fecal coliform was also found in all the samples except samples from site B (Ashomu). The presence of pathogenic microorganisms, most especially *Escherichia coli and Staphylococcus aureus* in hand pumped well water can be attributed to poor hygiene and sanitary practices around these sources of water.

According to WHO, no fecal coliform should be detected in any 100 mL of drinking water. From the result, it may be concluded that majority of the water samples appraised in this study except from sample site B (Ashomu), are not suitable for direct human consumption without prior treatment. The results of this study are similar to results of a previous study carried out by Akinyemi et al. (2006), that most well water at Sagamu are not microbiologically safe for drinking without additional treatment such as boiling or disinfection. Location of the hand-pumped well and environmental factors may be responsible for the level of contamination in some of the samples with higher number of total viable bacterial counts. Some domestic animals may also visit the site and when drinking, they lick the mouth of the hand-pumped well taps and defecate around the pump location. These activities could enhance bacterial spore to contaminate the water through the opening side.

The isolates from the water samples were further identified using molecular methods (PCR). A universal primer which targets different gene segments of various bacteria was used. While *Streptococcus pneumonia* was identified by the groES gene, *Staphylococcus aureus* was identified using the mecA gene. *E. coli* was identified using its plasmid and Klebsiella identified using the GapA gene. This method of identification has also been used by Hung *et al*. (2005) and Siri *et al*. (2011).

E. coli was isolated in 83.3 % of the sampled hand-pumped well water. The high density of *E. coli* in the drinking water sources may be due to untidy nature of the physical environment and proximity of some wells to toilets and refuse dump as observed particularly in sample site D (Safari village), where the hand-pumped well was located in student hostel, though the environment was clean and tidy, the hand-pumped well was close to a septic tank, which could account for the presence of *E. coli* and *Streptococcus* sp. in the water. The findings of this study are similar to earlier reports by Gwimbi, (2011) and Okorafor *et al*. (2012) in their study on physico-chemical and bacteriological characteristics of selected streams and boreholes in Akamkpa and Calabar Municipal and Maseru district Lesotho respectively where they reported high rate of coliforms contamination in all the water analyzed.

Other organisms like *Klebsiella oxytoca*, *Staphylococcus aureus* and *Streptococcus pneumoniae* were isolated from the water samples. *Staphylococcus* species are usually non-motile, catalase positive, gram-positive cocci. They are commonly present as parasites of man and they form part of bacteria flora of the skin, upper respiratory tract and intestinal tract. *Staphylococcus aureus* (a type species) is by far the most significant and pathogenic in the genus. It is carried in the nose of 20 – 40% of healthy individual (Ochei and kolhatkar, 2004). *Klebsiella pneumoniae* is the causative agent of chest infection and occasionally, severe broncho Pneumonia with lung abscesses (Cheesbrough, 2006).

5. CONCLUSION AND RECOMMENDATION

The result of this study suggests proof of hand pump well water contamination by fecal coliforms. Since majority of the well water analyzed were found to be contaminated by pathogens of fecal origin, they are consequently not fit for human consumption. Proper hand-pumped well location at a distance above 15 meters away from the septic tank is essential, public awareness on environment sanitation should be emphasis. Also hand-pumped well samples that do not meet the standard should be treated before consumption.

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