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Serodetection of Hepatitis C Virus and molecular identification of Dermatophytosis in People with Tattoo in Diyala Province

A Thesis

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Fulfilment of the Requirements for the Degree of Masters of Sciences
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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالَ تَعَالَى: ﴿وَأَصْبِرْ لِحُكْمِ رَبِّكَ فَإِنَّكَ بِأَعْيُنِنَا وَسَبِّحْ بِحَمْدِ رَبِّكَ
حِينَ تَقُومُ ﴿٤٨﴾ وَمِنَ اللَّيْلِ فَسَبِّحْهُ وَإِدْبَرَ النُّجُومِ ﴿٤٩﴾﴾

صدق الله العظيم

الطور: ٨٤ - ٩٤

Dedication

I dedicate My Thesis to :

The Great Woman.....

My Mother

The Source of Light in My Life.....

My Father

My lovely Brothers and Sisters

*The person who supported me during my life in
general and study in particular*

My Childhood Friend

Ruqaia

Rehab

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Summary

Hepatitis C Virus remains a significant risk worldwide. Tattooing is one of the routes of transmission of infection from an infected person to another. Tattooing is a permanent design created by injecting external dye into the dermis. Tinea contagious are dermatophyte-caused fungal skin diseases that may affect up to 20% of the global population. The presence of fungal infections in the tattoo needle is assumed to be the source of fungus contamination during tattooing. The study aimed to detection of the frequency of HCV in peoples with tattoos by Elisa assay and molecular identification of Dermatophytes sp. from peoples with tattoos in Diyala governorate.

A cross-sectional study was done in the period from the 1st of October 2020 until the 15th of February 2021 in department Dermatology of Baquba teaching hospital (Consulting clinic, premarital screening program, and periodic examination of hairdressing salons within the preventive health affairs). The study included 100 patients (43 males and 57 females) aged from 10-65 years old. After blood collection, an enzyme-linked immunosorbent assay (ELISA) test was performed to detect hepatitis C virus antibodies (IgG).

The results of this study showed that the frequency of Hepatitis C Virus 17% (17 out of 100) with significant differences ($p < 0.05$), for age group HCV positivity, constituted the highest percentage rate of age groups between (31-40 years) followed by (21-30 years) with percentages of (58.8% and 41.2%) respectively.



There was no significant differences between age groups and Hepatitis C virus infection in the study population ($p > 0.05$).

Depending on gender, it was found that males with HCV formed the highest percentage rate (76.50%) compared to females (23.50%), with highly significant differences ($p < 0.05$). Depending on the educational level, it was found that the secondary school graduates had a higher incidence of HCV (52.9%) compared to primary schools and college graduates (41.2%) with no significant differences ($p > 0.05$).

While for the diseases, it was found that people without diseases formed the highest percentage rate of tattoo carriers (64.9%) as compared to the presence of diseases with significant differences ($p < 0.05$).

The mycological diagnosis were conducted by direct microscopy and by culture the specimens on each Sabourand dextrose agar and potato dextrose agar supplemented with chloramphenicol and cycloheximide.

The identified dermatophytes were represented *Trichophyton* sp (12%) in which *T. mentagrophytes* (9.0%), *T. rubrum* (3.0%) and *Microsporum* (6.0%) in which *M. audouinii* (3.0%), *M. gypseum* (3.0%). While non dermatophytes sp. Represented, *Tinea nigra* (3.0%), other growth (29.0%), with significant differences ($P < 0.05$).

Depending on age groups, it was found that *T. mentagrophytes* constituted the highest infection rate (33.3%) in age groups (21-30 years) and *M. gypseum*; *T. rubrum* were found to have the highest infection rate (33.3%), in age groups (31-40) of each species. While, *M. audouinii* constituted the highest infection rate (66.7%) in age

groups (31-40 years) with significant differences ($p < 0.05$) between age groups .

Depending on gender, *T. rubrum* and *T. mentagrophytes* were found, they constituted the highest rates of infection in males (66.70% and 34.50%) respectively. In females, *M. audouinii*, *T. rubrum* and *T. mentagrophytes* formed the highest infection rates (100.00%, 100.00% and 77.80%) respectively with no significant differences ($p > 0.05$).

Depending on housing, *T. rubrum*, *T. mentagrophytes* were found the highest rates of infection in people living in Rural areas (66.70% and 44.40%) respectively. As for the people who live in Urban , *M. audouinii* and *M. gypseum* formed the highest rates of infection (66.70%) respectively. The differences between fungal infection and habitation were not significant ($p > 0.05$).

Depending on the educational level, *M. gypseum*, and *M. audouinii* were found, The highest rates of infection were among people those who graduated from secondary schools with (66.70%). The differences between fungal infection and educational level were not significant ($p > 0.05$).

Then, there was a DNA extraction of 7 dermatophytes sp. isolates by polymerase chain reaction PCR assay and detection of *subtilisin like protease* gene. The samples that have this gene were sent to sequencing. The results of PCR showing that 2 of 7 isolates have *subtilisin like protease* gene this gene responsible of pathogenesis of dermatophytes.



The Sequence of isolated *Trichophyton rubrum* was new sequence, and recorded in the Gene bank of NCBI.

Resistance to antifungal was noticed in Caspofungin and Fluconazole drugs.

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List of Abbreviations

Abbreviations	Meaning
aa	Amino acid
bp	Base pair
C	Core
CDC	Center for Diseases control
CLD	Chronic liver disease
DAA	Direct Acting Antiviral
E	Envelop
EIA	enzyme immunoassays
ELISA	Enzyme linked immunosorbent assays
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICTV	International Committee on Taxonomy of Viruses
IDU	Intravenous drug users
KOH	Potassium hydroxide
LHR	laser hair removal
LTR	laser tattoo removal
MSM	Men who have sex with men
Nt	Neclotides
ORF	Open reading frame
OT	Ocular tattoo
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar

PWID	People who inject drugs
RdRp	RNA-dependant RNA polymerase
RNA	Ribonucleic acid
SDA	Sabouraud Dextrose Agar
SDACC	Sabouraud Dextrose Agar with Chloramphenicol and Cycloheximide
SDS	sodium dodecyl sulfate
TRC	tattoo related complications
UTR	Untranslated regions

Chapter One

Introduction

1.1 Introduction

The hepatitis C virus (HCV), which is a common cause of acute and chronic hepatitis, is a major risk factor for liver cirrhosis and hepatocellular carcinoma. (Mrzljak *et al.*, 2021). In up to 80% of cases, HCV infections are chronic, resulting in about 71 million people worldwide suffering from chronic hepatitis C. (Behrendt *et al.*, 2019). Hepatitis C virus is one of the most commonly transmitted infectious agents by Blood transmitting, so, it still remains a considerable global health problem (Othman and Abbas, 2020).

The hepatitis C virus is a member of the Hepacivirus genus of the Flaviviridae family (Catanese *et al.*.,2013). The HCV genome consists of a single strand positive-sense ribonucleic acid RNA encoding a single polyprotein (Borgia *et al.*, 2018) .

The inability to mount an effective protective immune response and the high variability of HCV genotypes subtyped to 67 (Lanini *et al.*, 2016). Approximately 20% of incident reports, however, have no history of IDU or other parental exposure. (Behrendt *et al.*, 2019)

Despite the clinical sequel associated with HCV, its epidemiology and risk factors are poorly understood, especially in developing countries where intravenous drug abuse (IDU) is uncommon. Several studies documented that HCV is transmitted by blood transfusion and other percutaneous exposure and possibly sexual or household contact with HCV infected persons (Al-Kubaisy and Niazi, 2013). The presence of viral pathogens in the tattoo ink and needle is expected to be the cause of viral

contamination during tattooing. Cutaneous viral contaminations due to human HCV, HBV virus and HIV have been reported in association with tattooing (Temiz and Özlü, 2021). Infections with the HCV are pandemic, and the World Health Organization (WHO) estimates a world-wide prevalence of 3% in Middle Europe, approximately 1% of the population is infected, mostly with genotype 1 (85% in Austria). In developing countries, chronic hepatitis C is the most prominent cause for liver cirrhosis, hepatocellular carcinoma and liver transplantation.

Today, sporadic transmission is more prevalent, mostly in drug addicts via needle sharing, and seldom by needle-stick injuries in medical personnel, vertical transmission from mother to baby, tattooing, piercing, or razor sharin (Fallahian and Najafi, 2011) .

In incarcerated population, the prevalence of hepatitis C virus (HCV) is high, especially among those with a history of using inoculation drugs (Poulin *et al.*, 2018). Penetration of the skin barrier by needling opens the door to infection. Historically, there were concerns that tattooing would facilitate transmission of blood-borne infections such as hepatitis B and C, HIV, and syphilis. Modern sterile and hygienic standards have dramatically reduced this risk when tattoos are applied by licensed professionals (Farley, Van Hoover and Rademeyer, 2019)

The prevalence of tattooing is on the rise in the United States. A recent Harris poll reflects a significant increase in tattooing among adults in the last decade, with 1 in every 5 reporting one or more tattoos in 2012 (Braverman, 2012).

Tattooing refers to the practice of injecting ink into the skin using a special tool to create a permanent or long-lasting image, design or word (Jafari *et al.*, 2020). The presence of pigment in the dermis can be regarded as a tattoo, whether done on purpose or as a result of external trauma. Tattoos can be classified into five categories: professional, amateur, cosmetic, traumatic, and medical (Kurniadi *et al.*, 2020)

Tattooing is a well-known ancient artistic practice characterized by the intradermal placement of pigment, and it has increased in popularity over the past 20 years (Mrzljak *et al.*, 2021). Prison tattooing is of particular concern regarding the communication of blood-borne diseases since tattooing is normally performed in this environment using non-sterile devices, such as guitar strings, paper clips or stitching needles, which are generally purified by heating or using boiling water (Carney *et al.*, 2013)

In Iraq, there is a prevalence of viral hepatitis C in Iraqi cities such as in Baghdad (0.098%) (AL-Dafae *et al.*, 2020), Basra (2.8%) (Abdul-Jalil and Al-Asadi, 2016), and Dohuk 0.2% (Ramadhan, 2018). Another study showed that the prevalence was (13.9%) in Najaf governorate (Al-Nafakh *et al.*, 2019). Infection with the hepatitis C virus is on the rise among hemodialysis patients in Diyala Governorate was 7.8% (Khalaf and hussain 2021), and in Al-Anbar was 22.5% in Al Ramadi teaching hospital (Abdulwahab *et al.*, 2021).

Fungal contamination throughout tattooing is thought to be due to the incidence of fungal pathogens in the tattoo needle. Cases of superficial Dermatophyte infection infected with *Epidermophyton*

floccosum and *Trichophyton rubrum* have been reported in association with tattooing (Temiz and Özlü, 2021). Literature reports recommend the occurrence of *Tinea spp* .infection within a month of tattoo location (Yadav *et al.*, 2018).

Dermatophyte infections are among of the earliest known infections of mankind and are very common throughout the world (Shahid and Khan, 2016). The infections caused by these fungi are usually named after the infected part of the body rather than the infecting organism. Fungal infections can cause illness in immunosuppressed persons, mainly those suffering from transplants, chemotherapy, and human immunodeficiency virus-positive patients (Rouzaud *et al.*, 2015). Tinea infections are fungal infections of the skin caused by dermatophytes that may affect up to 20% of the global population. These infections may be further classified as *tinea corporis* (ringworm) and *tinea cruris* (jock itch) and are generally diagnosed by appearance and confirmed with microscopy or culture (Ray, 2017).

Dermatophytes are to referred as a unique group of superficial keratinophilic and filamentous fungi which have the ability to attack keratinized tissue of the skin, nails, and hair in humans and animals leading to dermatophytosis or tinea (ringworm) (Alkahafajii, 2014). Dermatophytosis is described either by the unusual range of skin surface part affected by the infection or by the uncommon amount of affected locations. However, the infection remains confined to the epidermis or associated keratinized structures such as nails (Mansouri *et al.* 2012). Dermatophytes are pathogenic fungi that can attack keratinized forrms and infect skin, hair, and nails of animals and humans (Xiao *et al.*, 2015)

A dermatophyte fungus has the ability to produce keratinase, which allows them to break down, and live on human keratin like skin, hair and nails (Shahid and Khan, 2016). The greatest valuable dermatophytes isolated in Europe are *T.rubrum*, *M.canis*, *T.mentagrophyte* var.*granulasum* and *T.verrucosum*. Numerous of these are supposed to have expanse from the Mediterranean Countries. Further dermatophytes such as *M.audouinii*, *T.soudenense* and *T.violaceum* that are endemic in Africa and Asia are now hardly ever isolated in Europe. In Asia *T.rubrum* and *T.mentagrophytes* are the greatest usually isolated pathogens, causing tinea pedis and tinea unguium (Menon and Routray, 2015)

They rely on keratin as a source of nutrient and cause its hydrolysis by releasing a number of enzymes such keratinase as a proteolytic enzyme of keratin protein which is very hard and strong (Sharma and Swati, 2012). Overall, these fungi live in humid parts of the body, low standards of living along with high humidity environments are contributing to the increased prevalence of these fungal infections (Blutfield *et al*, 2015). Dermatophytes infections are triggered by 40 fungal species which are classified into 3 genera; *Trichophyton*, *Microsporum* and *Epidermophyton* (Abdeen and El-Diasty, 2015). According to the unamorphic state, there are two species of *Epidermophyton*, 25 species of *Trichophyton* and 18 species of *Microsporum* (Mucoma, 2000; Sharma *et al.*, 2015).

1.2 Aims of study

The study aimed to:-

- 1- Detection of hepatitis C virus in patients with tattoo in Diyala province.
- 2- Studying the relationship between tattoo and infection rate of hepatitis C virus and correlation between HCV with age, gender, education, residence, other diseases.
- 3- Detection of the frequency and relationship of Dermatophytosis among tattoo patients.
- 4- The molecular identification of dermatophytes sp. Patients by using PCR and sequence.