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ORIGINAL ARTICLE

# ARMS as a Genotyping PCR, a New Technique for Detection of Liver Transplantation Recipients' C3FF, C3FS, C3SS Allotypes as Predisposing Factor For Post-Transplantation Infections

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## ABSTRACT

***Background****: Complement is a crucial branch of both non-specific and specific immune system. C3 is the third complement component which plays a protective role in viral infections. There are two co-dominant inherited variants or allotypes for C3: C3 fast (C3F) and C3 slow (C3S). C3F variant has been linked to multiple diseases production including infections among liver transplantation recipients.* ***Objectives****: To investigate the most common risk factors for post-liver transplantation infections especially pre-operative colonization. To identify types and rates of post-liver transplantation infections and their causing organisms. To identify C3 allotypes for both liver transplantation donors and recipients, and to correlate recipients' allotypes with post-transplantation infections.* ***Methodology:*** *This study is a prospective study; it was conducted from January 2017 to August 2019 on 64 chronic cirrhotic patients, experienced liver transplantation in the National Liver Institute ICU, Menoufia, Egypt, and their donors. Blood cultures and other samples were collected according to site of infection using standard Microbiological sample collection methods and bacterial isolated were identified by standard microbiological methods using VITEK2 Compact automated ID/AST instrument. CMV IgG and IgM were detected by ELISA method. DNA was extracted and the extract was used for detection of C3S and C3F alleles by using Amplification Refractory Mutation System (ARMS).* ***Results:*** *The most common cause for liver transplantation was HCV related liver cirrhosis (26.56%). Risk factors for post-liver transplantation were Pre-operative microbial colonization (100%) and long operative time (9.875±2.45 h). 39.8% of post-transplantation infections occurred during second week post-operatively and the commonest infections were drain infections (29.5%) and urinary tract infection (27.3%). 51.7% of liver transplantation recipients were C3FF, 32.8% were C3FS and 15.6% were C3SS. C3FF recipients showed increased relative risk to develop CMV (5.2) and bacterial & fungal infections (2.2) than other recipients' allotypes.* ***Conclusion****: A comprehensive infectious diseases workup including detection of C3 allotype of the candidate for liver transplantation should be done pre-operatively to early detect and treat infections which can improve the outcome of the operation dramatically and improve patients' life style. More studies should be done to find out if there is relation between donors' and recipients' C3 allotypes as currently no clear data still present.*

## INTRODUCTION

Liver transplantation procedure is major, long and complicated surgical operation done for severely ill patients[1]. One of the most crucial problems affecting morbidity and mortality after liver transplantation is infection. Risk factor for infection following liver transplantation is multifactorial and every factor need to be investigated to decline all the risk factors related to post-transplantation infection whether it is donor or recipient related. There are three groups of risk factors: a) Transplantation-related factors: ischemia reperfusion damage, volume of blood transfusion intra-operative, type of biliary drainage, type of immunosuppressive taken, graft rejection, extended stay in intensive care unit along with dialysis / ventilation, repeated transplantation, antimicrobial regimens including antiviral regimens. b) Donor-related risk factors: Bacterial infections, extended stay in intensive care unit, status of the donor's liver (e.g. steatosis), and viral infection. c) recipient-related risk factors are MELD score >30, malnourishment, kidney failure, acute


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hepatic failure, infections/colonization and immunological status against viruses such as Cytomegalovirus[2].

Cirrhotic patients usually suffer from sepsis mostly due to dysfunctional immunological defensive mechanisms towards bacterial, viral and fungal infections. Most of infections develop within the first month post-transplantation and they are mainly caused by aerobic gram-negative bacteria (GNB) and yeasts which arise endogenously due to colonization of oropharynx, stomach and bowel. Also invasive fungal and cytomegalovirus infections are common infections causing significant morbidity and mortality in transplant recipient which can be controlled by implementing certain prophylactic regimens[1].

Complement as recognized a pivotal branch of both non-specific and specific immune system, has three well-recognized physiologic functions, first is defense of host against infections, second is crossing point between the innate and adaptive immunity, and finally immune complexes clearance or cells apoptosis[3].

Complement system is an enzymatic system consists of 30 membrane bound and soluble proteins synthesized in the liver occur in inactive form in both serum and tissue fluids of human and warm blooded animals but not in CSF and urine. Complement system has dual functions; host defense and an inflammatory response which are integral part of whole immune response. Complement system cross-links innate and adaptive immune responses via different mechanisms including regulation of antibody effector mechanism and T-cell function modulation causing some cellular responses such as apoptosis, opsonization and amplification of inflammatory reaction. C3 is the third complement component which plays a protective role in viral infections where c3b, product of c3 cleavage, binds to activator surface initiating immune response trying to contain infection where antibody response is not initiated yet[4].

It has been shown that polymorphisms of C3 complement component is associated with liability to different diseases like systemic lupus erythematosus, age related macular degeneration (ARMD) and more vulnerability to infections caused by encapsulated pyogenic bacteria like bacteremia, pneumonia, meningitis and osteomyelitis. While many viruses are targeting C3 as it is the core component for activation of the three complement system pathways[4].

There are two codominant inherited variants or allotypes for human complement component C3: C3 fast (C3F) and C3 slow (C3S). The polymorphism is dependent on a C to G nucleotide change located at protein position 80 in C3b fragment produced after proteolytic cleavage of C3 which gives glycine in C3F and arginine C3S[5].

The two allelic variants S (slow) and F (fast) of C3 caused by single base substitution are so called due to differential mobility on high voltage agarose gel electrophoresis of the resultant proteins driven from serum[6]. The two alleles give three phenotypes C3SS, C3FS and C3FF that form 98% of whole C3 phenotypes. 44 The molecular difference between the two variants is mainly due to SNP rs2230199 which is found in nucleotide position 394 (C-G), causing amino acid substitution.45 There are other rare C3 variants published in some literatures. 46 The frequency of C3F alleles vary significantly amongst races: 20% in whites, 47 5% in blacks and 1% in Asians.44[7].

Some studies investigated the association of C3 allotypes with the production of panel of diseases and concluded that there is pathologic role for C3F variant with multiple diseases including; renal diseases (Mesangiocapillary nephritis, Focal and segmental glomerulosclerosis, atypical haemolytic uraemic syndrome and dense deposit disease), Endocrinological diseases (Metabolic syndrome), Gastrointestinal diseases (Crohn's disease and colitis ulcerosa), Rheumatological diseases (systemic vasculitis and systemic lupus erythematosus), Haematological diseases (Non-hodgkin lymphoma), Dermatological diseases (atopic dermatitis, Psoriasis and partial lipodystrophy), Pulmonary diseases (asthma, chronic obstructive pulmonary disease, tuberculosis and lung cancer), neurological diseases (Ischaemic stroke), Cardiovascular diseases (hypertension, myocardial infarction and Chagas disease cardiomyopathy) and even transplantation (graft rejection)[7]. This was explained as the C3F binds to factor H (soluble complement regulator) resulting in more powerful amplification of the alternative pathway[5].

## METHODOLOGY

This study is a prospective study; it was conducted from January 2017 to August 2019 on 64 chronic cirrhotic patients and their donors. Patients experienced liver transplantation in the National Liver Institute ICU, Menoufia, Egypt. Age ranged between 8 – 60 years. The study protocol was approved by ethics committee of the National Liver Institute.

Blood samples were collected; 5 ml in EDTA tubes for PCR assay and preserved at -80 C$^{o}$ till the time of PCR assay. Another 5 ml were extracted in plain tubes for CMV Abs detection.

Blood cultures were collected from blood-stream infection suspected patients, blood was taken under complete aseptic condition during period of fever and 2 blood cultures were done with at least 1 hr interval where both aerobic and anaerobic blood culture bottles were inoculated each with 8 ml peripheral blood and incubated in BACT/ALERT system. Positive culture bottles were subcultures on routine Microbiology media including blood agar and MacConkey agar (Oxoid, UK) and incubated at 37 C$^{o}$ for 24 hours. Bacterial isolates

were identified by standard microbiological methods using VITEK2 Compact automated ID/AST instrument. Cultures considered negative and discarded after 5 days incubation without giving positive alarm.

Other samples were collected according to site of infection using standard Microbiological sample collection methods and bacterial isolated were identified by standard microbiological methods using VITEK2 Compact automated ID/AST instrument.

For colonization detection, nasal, throat and pharyngeal swabs were collected using sterile cotton swabs and stool cultures were done by collecting stool in sterile wide-mouthed screw-capped bottles. Isolated bacterial species were identified by standard microbiological methods using VITEK2 Compact automated ID/AST instrument.

CMV IgG and IgM were detected in liver transplant recipients' sera using test kit from Sigma Diagnostics (USA) and operated according to manufacturer instructions by using Enzyme-linked immune sorbent assays (ELISA) and results were interpreted according to van der et al.[8] and Nielsen et al.[9]. Where, blood samples were collected in dry clean plain tubes and left to clot for 30 minutes at room temperature. Then tubes were centrifuged for 5 minutes at 4000 rpm, and the serum was used for doing the test.

**Genomic DNA extraction:**

DNA extraction step was done on the recipient and donors EDTA blood samples stored at -80 C$^{o}$ after being thawed. DNA was purified from peripheral blood mononuclear cells (PBMCs) using QIAamp DNA mini Kit (Qiagen, Hilden, Germany).

*DNA amplification for detection of C3S and C3F alleles by PCR:*

DNA extract was used for detection of C3S and C3F alleles by using Amplification Refractory Mutation System (ARMS) PCR technique. This technique mandates that only terminal 3'-nucleotide of the primer to be allele specific. So the primer is being synthesized in two forms where the normal C3S primer form is refractory to hybrid with a mutant C3F DNA template and so on. Intended introduction of additional misalliances near the 3' end of normal primers don't not allow for nonspecific amplification to continue, so this method is preferred as it looks more suitable for genotyping than endonuclease enzyme digestion which contains two performed reactions.

*Primers used for C3 ARMS reaction:*

We used three primes; one reverse primer in common (5′-TGTTGACCATGACCGTCCGGCCCACGGTA-3′) and two forward specific primers, for C3S it was (5′-CCAACAGGGAGTTCAAGTCAAAAGGTGG-3′) and for C3F it was (5′-CCAACAGGGAGTTCAAGTCAGAAAAG GTGC-3′). Contents of the reaction included: 25 µL master mix containing 100 ng of extracted genomic DNA, 0.6 µM of every primer, 3 mM of MgCl2, 0.32 mM of every dNTP and 1.5 U of Taq polymerase. The reaction was performed by a thermocycler (ThermoFisher, USA) under the following conditions: 2 min at 94°C for primary denaturation, followed by 33 amplification cycles of 30 s at 94°C denaturation, 1 min at 50°C primer annealing and 1 min at 72°C for extension. Finally, one extension cycle was done for 10 min at 72°C. PCR products were separated on 2% agarose gel electrophoresis using ethidium bromide staining at 80V for 2h and visualized under UV light. Genotypes were determined for both donors and recipients[10].

*DNA amplification for detection of CMV:*

It was done by using quantitative real-time PCR using a light Cycler H Instrument (Roche Diagnostics, Meylan, France) with the QuantiTect Probe PCR Kit (Qiagen) and Taqman probes (6FAM-CGCGAGACCGTGGAACTGCG-TAMRA), forward primer (5′GCAGCCACGGGATCGTACT-3′) and reverse primer (5′GGCTTTTACCTCACACGAGCATT-3′) after being extracted using a Qiagen DNA extraction kit (Qiagen, Valencia, CA, USA) following manufacturer's instructions[11].

## RESULTS

Of the 64 liver transplantation recipient patients included in this study 49 (76.6 %) were male patients and 15 (23.4 %) where female patients. 50 (78.1 %) of the liver transplantation donors were males and 14 (1.9 %) were females with no statistical significant difference between the two groups. The age of liver transplantation recipient patients ranged between 8 - 60 years with mean age 36.4±15.4 while the age of liver transplantation donors ranged between 19-44 years with mean age 33.3±7.3 with no statistical significant difference between the two groups (table 1).

**Table 1 Demographic Data of Donors and Recipients**

| Demographic Data | | NO (%) | P value |
|---|---|---|---|
| Recipient mean age (years) | | 36.4±15.4 | 0.1481 |
| Donor mean age (years) | | 33.3±7.3 | |
| Recipient gender | Males | 49 (76.6 %) | 0.832771 |
| | Females | 15 (23.4 %) | |
| Donor gender | Males | 50 (78.1 %) | |
| | Females | 14 (1.9 %) | |

Regarding the indications for liver transplantation the highest indication was HCV related liver cirrhosis 17 cases (26.56 %) followed by autoimmune hepatitis and progressive familial intrahepatic cholestasis 12 cases each (18.75 %) then cryptogenic liver cirrhosis 9

cases (14.06 %) and HBV related liver cirrhosis 6 cases (9.37 %). While fewer cases caused by HCC on top of HCV related cirrhosis only 5 cases (7.81 %) and the least cause was primary biliary cirrhosis only 3 cases (4.69 %) (table 2).

**Table 2 Indications of liver transplantation**

| Cause | No. | % |
|---|---|---|
| HCV related liver cirrhosis | 17 | 26.56 |
| Autoimmune hepatitis (AIH) | 12 | 18.75 |
| Progressive familial intrahepatic cholestasis (PFIC) | 12 | 18.75 |
| Cryptogenic liver cirrhosis | 9 | 14.06 |
| HBV related liver cirrhosis | 6 | 9.37 |
| HCC on top of HCV related cirrhosis | 5 | 7.81 |
| Primary biliary cirrhosis | 3 | 4.69 |
| Total | 64 | 100 |

Among the risk factors of post-liver transplantation infections is pre-operative infections and colonization which was found in all 64 (100%) patients and long operative time which was 9.87±2.45 hour (table 3). In our study the most colonizing organism was k pneumoniae found in 37 (41.5%) patients then Vancomycin-resistant Enterococci (VRE) isolated from 24 (27.6%) patients. MRSA colonized 17 (19.5%) patients. *Candida albicans* (*C albicans*) isolated only from 6 (6.89%) patients when *Klebseilla oxytoca* was the least colonizing microbe it was isolated from only 4 (4.51%) patients (table 4).

**Table 3 Operative and pre-operative risk factors predisposing to post-transplantation infections**

| Risk Factors | No of Cases (n=64) |
|---|---|
| Pre-operative microbial colonization | 64 (100 %) |
| Length of operative time (mean±SD) | 9.875±2.45 |

**Table 4 Distribution of microbial species causing pre-operative colonization**

| Microbial species | No | % |
|---|---|---|
| *Klebseilla pneumoniae* | 37 | 41.5 |
| *Klebseilla oxytoca* | 4 | 4.51 |
| MRSA | 17 | 19.5 |
| VRE | 24 | 27.6 |
| *Candida albicans* | 6 | 6.89 |
| Total | 87 | 100 |

* Some patients were colonized with multiple microbes

Liver transplant recipient patients were followed for 1 year post-operatively to investigate post-transplantation prognosis. 40 (62.5%) patients survived and 24 (37.5%) died during the first 3 months; 9 (37.5%) died in the first month post-operative, 6 (25%) in the second month and 9 (37.5%) in the third month. The most common cause of death was Hemodynamic Instability where it was the cause of death in 16 (39.02%) patients where the second cause of death was sepsis 12 (29.3%) patients then graft loss after recurrent hepatic artery thrombosis which caused death of 7 (17.07%) patients. The last and least cause was Graft Dysfunction which happened in 6 (14.7%) patients. Some developed post-transplantation infections viral and bacterial. 13 (20.3%) got infected with CMV infection, 13 (20.3%) developed recurrent HCV infection and 50 (78.1%) got bacterial and fungal infections (table 5).

**Table 5 post- transplantation prognosis**

| Prognostic item | Patients No & % |
|---|---|
| Survival (n=64) | |
| Survived* | 40 (62.5) |
| Died | 24 (37.5) |
| Survival period (n=24) | |
| 1 month | 9 (37.5) |
| 2 month | 6 (25) |
| 3 month | 9 (37.5) |
| Cause of death (n=41) | |
| Sepsis | 12 (29.3) |
| Hemodynamic Instability | 16 (39.02) |
| Graft Loss after Recurrent HAT** | 7 (17.07) |
| Graft Dysfunction | 6 (14.7) |
| CMV infection | 13 (20.3 ) |
| HCV recurrence | 7 (10.9) |
| Bacterial & fungal infections | 50 (78.1) |

* Survived: Patients were followed for 1 year post-operatively
** HAT: Hepatic artery thrombosis

During the first week post-operatively the most common infections occurred were pneumonia 9 infection cases (37.5%) and UTI 7 infection cases (29.1%) followed by drain infection and catheter-associated blood stream infection (CLABSI) 4 infection cases each (16.7%). While in the second week the most common infections occurred were drain infection 16 infection cases (45.7%) and CLABSI 10 infection cases (28.6%) then UTI 5 infection cases (14.2%) and pneumonia 3 infection cases (8.6%) while cholangitis occurred only in 1 infection case (2.9%). Also in the third week the most common infection was drain infection 6 infection cases (50%) followed by pneumonia 3 infection cases (5%), while for UTI, cholangitis and SBP (spontaneous bacterial peritonitis)

there were only one infection case for each (8.3%). In the fourth week the most common infection was UTI 11 infection cases (64.8%) followed by CLABSI and SBP 3 infection cases each (17.6%). Where the total number of infections in the first week was 24/88 (27.3%), in the second week was 35/88 (39.8%), in the third week was 12/88 (13.6%) and 17/88 (19.3%) in the fourth week, with high statistical significant difference between the four weeks regarding the type of infections (table 6). Regarding the microbial species causing post-transplantation infections according to type of infection, it is shown in (table 7).

**Table 6 distribution of types of post-operative infections according to time of onset:**

| Type of infection | 1st Week Post-OP (no. & %) | 2nd Week Post-OP (no. & %) | 3rd Week Post-Op (no. & %) | 4th Week Post-Op (no. & %) | Total (no. & %) |
|---|---|---|---|---|---|
| Drain infection | 4 (16.7) | 16 (45.7) | 6 (50) | 0 (0) | 26 (29.5) |
| CLABSI | 4(16.7) | 10 (28.6) | 0 (0) | 3 (17.6) | 17 (19.3) |
| UTI | 7 (29.1) | 5 (14.2) | 1 (8.3) | 11 (64.8) | 24 (27.3) |
| Pneumonia | 9 (37.5) | 3 (8.6) | 3 (25) | 0 (0) | 15 (17.1) |
| Cholangitis | 0 (0) | 1 (2.9) | 1 (8.3) | 0 (0) | 2 (2.3) |
| SBP | 0 (0) | 0 (0) | 1 (8.3) | 3 (17.6) | 4 (4.5) |
| Total | 24 (27.3) | 35 (39.8) | 12 (13.6) | 17 (19.3) | 88 (100) |
| P Value | < 0.001 | | | | |

**Table 7 distribution of microbial species causing post-transplantation infections according to type of infection:**

| Bacterial isolate | Drain infection | CLABSI | UTI | Pneumonia | Cholangitis | SBP | Total |
|---|---|---|---|---|---|---|---|
| MRSA | 0 | 7 | 13 | 3 | 0 | 3 | 26 |
| *S. aureus* | 0 | 4 | 3 | 0 | 0 | 0 | 7 |
| *S. epidermidis* | 3 | 0 | 0 | 0 | 0 | 0 | 3 |
| *Strep pneumoniae* | 3 | 3 | 0 | 6 | 0 | 0 | 12 |
| *E. faecalis* | 3 | 0 | 0 | 0 | 0 | 1 | 4 |
| *K pneumoniae* | 8 | 0 | 5 | 6 | 2 | 0 | 21 |
| *Ps. aeruginosa* | 3 | 0 | 0 | 0 | 0 | 0 | 3 |
| *p. mirabilis* | 0 | 3 | 0 | 0 | 0 | 0 | 3 |
| *A. baumanii* | 0 | 0 | 0 | 3 | 0 | 0 | 3 |
| *C. albicans* | 6 | 0 | 3 | 0 | 0 | 0 | 9 |
| Total | 26 | 17 | 24 | 18* | 2 | 4 | 91 |

*****Three pneumonia cases were mixed infections

C3 allotypes were detected and amplified by using ARMS PCR a more specific PCR technique than restriction endonuclease digestion. Two PCR reactions were done one for detection of C3S allele and one for detection of C3F allele. Then the two amplification products for every sample were injected in agarose gel electrophoresis wells, then the allelic profile detected as following: if a sample is positive for C3F only it is C3FF, if it is positive for C3S only so it is C3SS, if it is positive for both C3S & C3F so it is C3FS. In our study 33 (51.7%) of the liver transplantation recipients had C3FF allotype, 21 (32.8%) were C3FS and only 10 (15.6%) were C3SS as shown in (table 8) and (figure 1).

**Table 8: genotypic frequencies of donors and recipients C3 allotypic variants**

| Allotypic Variant | C3SS Donor | C3FS Donor | C3FF Donor | Total |
|---|---|---|---|---|
| C3SS Recipient (No. & %) | 2 (20) | 2 (20) | 6 (60) | 10 (15.6) |
| C3FS Recipient (No. & %) | 3 (37.5) | 9 (31) | 9 (33.3) | 21 (32.8) |
| C3FF Recipient (No. & %) | 3 (37.5) | 18 (62.1) | 12 (44.5) | 33 (51.7) |
| Total (No. & %) | 8 (12.5) | 29 (45.3) | 27(42.2) | 64 (100) |

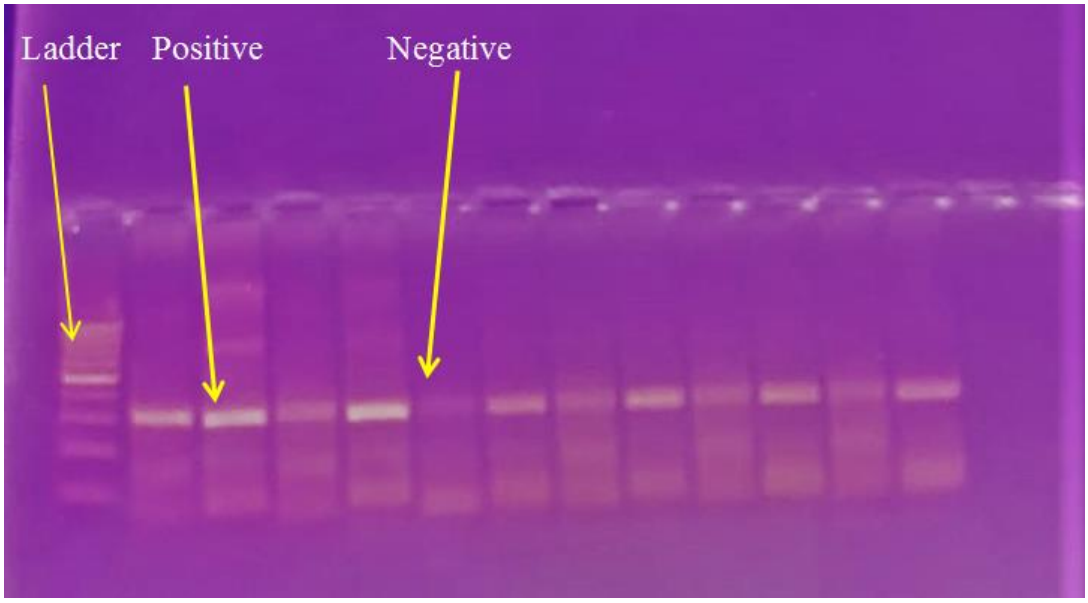


**Figure 1:** ARMS PCR for detection of amplified allotypes of C3 by agarose gel electrophoresis. Every sample is inoculated in two wells, one for C3F amplification product and the other for C3S amplification product. Then according to the presence of each gene the sample is defined as C3FF, C3SF or C3SS

By comparing liver transplantation recipients' allotypes with post-operative infection rate by calculating relative risk (RR) between recipients with allelic profile C3FF (as the claimed higher post-operative complications and infections rate) and C3FS and C3SS (as a less risky group), it was revealed that the recipients with C3FF allotype were 5.2 times more likely to have CMV infection compared to C3FS & C3SS allotype recipients with high statistical significance difference (P value =0.007). Recipients with C3FF allotype is 0.16 times less likely to get recurrent HCV infection compared to C3FS & C3SS allotype recipients with statistical significance difference (P value =0.015). Recipients with C3FF allotype is 2.2 times more likely to have CMV infection compared to C3FS & C3SS allotype recipients with high statistical significance difference (P value =0.001) as shown in (table 9).

**Table 9 Incidence of post-transplantation infections among different C3 allotypes:**

| Post-transplantation infections | C3 FF allotype (n=33) | C3 FS & C3 SS allotypes (n=31) | Relative risk (RR) | P value |
|---|---|---|---|---|
| CMV infection | 11 | 2 | 5.2 | 0.007 |
| HCV recurrence | 1 | 6 | 0.16 | 0.015 |
| Bacterial & fungal infections | 33 | 14 | 2.2 | 0.001 |

 For statistical analysis C3 FS &C3 SS allotypes are grouped together

## DISCUSSION

Complement as recognized a pivotal branch of both non-specific and specific immune system, has three well-recognized physiological functions, first is defense of host against infections, second is crossing point between the innate and adaptive immunity, and finally immune complexes clearance or cells apoptosis[3]. One of the common polymorphisms in C3, primarily identified from the electrophoretic mobility is C3S/F, referring to migration slow/fast[12]. Evidence from in vitro experiments for actual functional difference between allotypes C3 S/F allotypes is still not clear and not surely confirmed. However, some experiments showed that C3F coated RBCs had more obvious rosetting with more mononuclear cells in peripheral blood than the RBCs coated with C3S. Other experiments showed that there is significant difference regarding the hemolytic activity of C3S on sensitized sheep RBCs and there were no significant differences between the two alleles regarding the uptake of complement on sheep RBCs, converting to inactive c3b form and the ability to convert the already formed immune-complexes to soluble form. In contrast, other experiments proved that there are differences between the two alleles C3S/C3F in the long term outcome of different diseases where diseases evidence with C3F is higher[13].

Our study was conducted on chosen compatible groups with no significant difference in age or sex. The study was investigating risk factors for post-operative infections after liver transplantation. As the study was conducted in national liver institute, Menoufia so the

most common indication for liver transplantation was HCV infection due to known higher incidence rate of HCV infection among Menoufia population where 26.56 % of liver transplant patients were infected with HCV. One of the risk factors for post-operative infections is pre-operative infections and colonization and prolonged operative time. In our study 100 % of liver transplant recipients included showed pre-operative infections or colonization and average operative time 9.87±2.45 hour where *Klebseilla pneumonia* (41.5 %) and VRE (27.6 %) were the most commonly colonizing bacteria. A result matching what was stated by Hoek et al.[2]. Hernandez et al.[12] claimed that most of bacterial infections happened during the first month after liver transplantation, which is matching with our findings where 88 % of liver transplant patients developed post-operative infections during the first month post-operatively with peak of infections (35 %) occurred during the second week post-operatively.

Twenty-four (37.5%) out of the 64 liver transplanted patients died during the first 3 months post-operatively, where the most common cause of death was hemodynamic instability 16/64 (39.02%) followed by sepsis 12/64 (29.3%), a finding that to some-extent resembling findings of Zhang et al.[15] who found that the most common cause of death was multi-organ failure with circulatory failure.

About 70 % of liver transplantation recipients included in the study experienced bacterial and fungal infections, and some got viral infection by CMV and other suffered from recurrence of HCV infection. These results are matching with Hernandez et al [14], study where it is mentioned that bacterial infections are most common infections after liver transplantation occurring in about 70 % of patients followed by viral infections, which may be due to use of immunosuppressive drugs plus other risk factors. In our study most of bacterial and fungal infections occurred during the second week post-operatively 35 (39.8%) with high statistical difference, where drain infection was the most common post-operative infection 26/88 post-operative infections (29.5%) followed by UTI 24/88 post-operative infections (27.3%), with the most prevalent organism MRSA (isolated form 26 patients) followed by *K pneumoniae* (isolated from 24 patients).

In our study we used ARMS PCR technique a more accurate methodology for target detection. We used it to detect C3 allotypes CEFF, CEFS, and C3SS allotypes in both donors and recipients. In liver transplantation recipients the most predominant allotype variant was C3FF, then C3FS and the least was C3SS, while in the donors the most predominant allotype variant was C3FS, then C3FF and the least was C3SS. By comparing risk rate between recipients with allotype C3FF in one group and recipients with allotypes C3FS and C3SS in other group to prove the claim of Hervás et al.,[5] that C3FF allotype is associated with higher risk of post-operative infections and the risk rate was calculated which showed that C3FF is definitely associated with higher post-operative infections where it was found that recipients with C3FF allotype were 5.2 times more likely to get CMV infection and 2.2 times more likely to have CMV infection compared to C3FS & C3SS allotype recipients with high statistical significance difference. BUT this was not true regarding HCV recurrent infection where it was found that C3FF allotype recipients were 0.16 times less likely to get recurrence of HCV infection than C3SS allotype recipients with statistical significance difference.

## CONCLUSION

There are limited researchers studied the relation of C3 allotypes directly with post-transplantation infection rate although it is informative and easy to do test which can predict the outcome of liver transplantation operation which is a great complex expensive surgery.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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