ASSESSING NUCLEIC ACID TESTING VERSUS ELISA FOR BLOOD VIRUSES DETECTION IN SOME BLOOD BANKS

Submitted By

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M.B.B.Ch., Faculty of Medicine, Ain Shams University, 2003

Master in Clinical Pathology, Faculty of Medicine, Ain Shams University, 2009

A Thesis Submitted in Partial Fulfillment

Of

The Requirement for the Doctor of Philosophy Degree

In

Environmental Sciences

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APPROVAL SHEET

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Nanis Salah

ABSTRACT

INTRODUCTION: Transfusion-transmissible viral infections, such as hepatitis C, hepatitis B, and human immunodeficiency viruses, remain a major public health problem in developing countries. Nucleic acid testing is a molecular technique for screening blood donations to reduce the risk of transfusion transmitted infections in the recipients, thus providing an additional layer of blood safety. The ABO blood group has been previously found to be associated with the risk of several malignancies, including gastric cancer, pancreatic cancer, ovarian and skin cancer**.** Many studies have been performed to determine relationship between infectious diseases and blood groups**. AIM OF THE WORK**: To assess the importance of implementing NAT assay to detect donors during window period which are not detected with Enzyme-linked immunoassay and to distinguish the possible relation of viral infection with ABO blood groups and Rh system (if any). **SUBJECTS AND METHODS**: This cross sectional study was conducted at the Egyptian Abbassia regional Blood Transfusion center at Abbassia, Cairo. Blood donation collected from 10000 voluntary donors from January 2016 to June 2016 and tested with both ID NAT and ELISA assays for HBV, HCV and HI,.also ABO group, Rh type testing. **RESULTS**: NAT testing has the potential to detect viral nucleic acids of HIV 1-2, HBV, and HCV earlier than current screening methods, also hepatitis C and B were found to be higher in donors who has blood group O (43.3%, 44%) \and lowest in donors who has blood group AB (6.7%, 3.2%) **CONCLUSION**: NAT screening for three viruses has improved blood safety.. Also seroprevalence of HBs Ag and HCV Ab were found to be higher in donors who has blood group O and lowest in blood group AB donors, while the distribution of Rh in hepatitis infections was higher between Rh positive donors.

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List of abbreviations

Ab	: Antibody
AE	: Acridinium ester
Ag	: Antigen
AIDS	: Acquired immunodeficiency diseases
ALT	: Alanine aminotransferase
Anti- HBe	: HBV entire antibody
Anti-HBc	: HBV core antibody
Anti-HBs	: HBV surface antibody
Anti-HCV	: HCV antibody
Anti-HIV	: HIV antibody
bDNA	: Branched DNA
BTS	: Blood transfusion service
CDC	: Center for Disease Prevention &control
CLIAs	: Chemiluminescent immunoassays
DKA	: Dual Kinetic Assay
DNA	: Deoxyribose nucleic acid
EIAs	: Enzyme immunoassays
ELISA	: Enzyme-linked immunoassay
Gp	: Glycoprotein
HA assay	: Haemagglutination assay
HAV	: Hepatitis A virus
HBcAg	: Hepatitis B core antigen
HBeAg	: HBV entire antigen
HBsAg	: Hepatitis B surface antigen
HBV	: Hepatitis B virus

List of abbreviations*(Cont..)*

HCV	: Hepatitis C virus
HIV	: Human immunodeficiency virus
HPA	: hybridization protection assay
IAS	: Immunoassays
IC	: Internal control
ICTV	: International Committee on Taxonomy of Viruses
ID NAT	: Individual NAT
ISBT	: International Society of Blood Transfusion
MMWR	: Mortality and morbidity weekly report
MP NAT	: Mini pool NAT
MP	: Mini pool
NANB	: Non-A, non-B
NASBA	: Nucleic acid sequence based amplification
NAT	: Nucleic acid amplification technology
NBTC	: National Blood Transfusion Center
OBI	: Occult HBV infection
PA assays	: Polyagglutination assays
PCR	: Polymerase Chain Reaction
RBCs	: Red blood cells
Rh	: Rhesus
RLU	: Reported as Relative Light Units
RNA	: Ribose nucleic acid
RNAP	: RNA polymerase
SPSS	: Statistic package for social science program
TTIs	: Transfusion transmitted infections

List of abbreviations*(Cont..)*

TMA : Transcriptation mediated amplification

TTVs : Transfusion transmitted viruses

WB : Western blot

WHA58 : WHO Health Assembly 58

WHO : World Health Organization

WNV : West Nile Virus

WP : Window period

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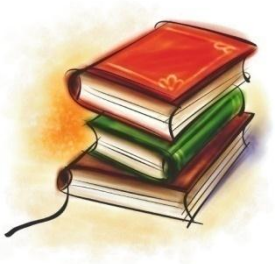

Introduction

Introduction

The goal of any transfusion service is to provide adequate and safe blood and blood products that meet the needs of patients. Transfusion transmitted infections (TTIS) is a recognized complication of blood transfusion and blood products. Many of these infectious agents may cause lifetime morbidity and/or mortality. The three major TTIs of viral origin associated with blood transfusion are human immunodeficiency virus (HIV), hepatitis C virus (HCV) and hepatitis B virus (HBV) ***(Gwarzo, 2009).***

Nucleic acid testing (NAT) is a molecular technique for screening blood donations to reduce the risk of TTIs in the recipients, thus providing an additional layer of blood safety ***(Roth et al., 2012).***

NAT technique is highly sensitive and specific for viral nucleic acids. It is based on amplification of targeted regions of viral ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). It detects them earlier than the other screening methods thus, narrowing the window period of HIV, HBV and HCV infections. NAT also adds the benefit of resolving false reactive donations on serological methods which is very important for donor notification and counseling ***(Yaseen et al., 2013).***

In the last few decades through an awareness of TTIs, a majority of countries have mandated serology based blood screening assays for HIV, HCV, and HBV. However, despite improved serological assays, the transfusion transmission of HTV, HCV, and HBV continues, primarily due to release of serology negative units that are infectious because of the window period (WP) ***(Shyamala, 2014).*** The WP is that period of time from infection to the time of detection by a given blood screening assay ***(Weusten et al., 2011).***

During this period, the risk of infection in donated blood can be missed by the immunoassay testing. These undetected WP infections

are responsible for most of the transfusion transmission of these viruses ***(Chigurupati and Murthy, 2015).*** NAT shortens this window period, thereby offering blood centers a much higher sensitivity for detecting viral infections. For example, with serology tests, it takes about two months after infection for anti-HCV antibodies to be detected, while NAT testing can detect HCV RNA in about five days after infection ***(Agarwal et al. 2013).***

NAT is a highly sensitive and advanced technique which has reduced the WP of HBV, HCV, HIV but it is highly technically demanding, involving issues of high costs, dedicated infrastructure facility, equipments, consumables and technical expertise. The need for NAT depends on the prevalence and incidence rate of infections in blood donor population, available resources and the evidence of benefit added when combined with serology tests. Hence the decision of starting NAT should be considered when basic quality assured blood transfusion system is already in place such as volunteer base for blood donation, provision of donor self-deferral, donor notification and counseling along with quality assured sensitive serological methods for testing TTIs ***(Hans and Marwaha, 2014).***

The ABO blood group has been previously found to be associated with the risk of several malignancies, including gastric cancer, pancreatic cancer, epithelial ovarian and skin cancer ***(Shim et al., 2015).***

Many studies have been performed to determine relationship between infectious diseases and blood groups ***(Aljooani et al., 2012).***

Among infectious disease, HIV, and Hepatitis viruses are of great concern because of their prolonged viraemia and carrier or latent state. They also cause fatal, chronic and life-threatening disorders ***(Ali and Fathallah, 2014).***