**Screening blood TTVs seronegative donors for variant Creutzfeldt-Jakob Disease Prion is not justifiable in Nigeria**

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**ABSTRACT**

The study was done to determine the prevalence of Variant Creutzfeldt-Jakob disease prion among blood donors in Owo. The blood samples were collected from blood donorscertifed fit on account of negativity for HIV, HBsAg and HCV; at Federal Medical Centre Owo. Sera of participants were analysed using PRNP ELISA kit. Structured questionnaire was used to obtain demographic characteristic and other relevant information for the study. Out of the ninety blood donors screened for variant Creutzfeldt –Jakob disease prion none was found to be seropositive giving overall prevalence rate of 0%. Including screening for variant Creutzfeldt –Jakob disease in the blood safety policy of Nigeria is not advised, as of now. Surveillance should be sustained to review the policy, as the world is a global village

***Keywords****: variant creutzfeldt-jakob disease, prion, blood safety, surveillance, TTV.*

**INTRODUCTION**

Blood transfusion is an integral part of laboratory medicine, also carries the risk of transfusion transmissible infection such as transmissible spongiform encephalopathies of human measuring their severity. World Health Organization has recommended pre-transfusion blood test as mandatory, where data exist to justify same. These diseases are capable of causing significant mortality, morbidity along with financial burden for both the affected person and country. With every one unit of blood transfusion there is a 1% chance of transfusion related complications including transfusion transmitted infections (Swapan *et al*., 2012).

The provision of safe and efficacious blood and blood component for transfusion involves a number of processes, from selection of blood donors and the collection, processing and testing of blood donation to the testing of patient samples, the issue of compatible blood and its administration to the patient. There is a risk of error in each process in this transfusion chain and a failure at any of these stages can have serious implication for the recipient of blood and blood products. Thus, while blood transfusion can be life-saving, there are associated risks, particularly the transmission of blood-borne infections (Adegoke and Akanni, 2011).

In humans, the most common transmissible spongiform encephalopathy (TSE) is called Creutzfeldt-Jakob disease (CJD). Creutzfeldt-Jakob disease is rare with a worldwide incidence of one case per million. Humans who develop this disease will slowly lose the ability to think and to move properly and will suffer from memory loss and progressive brain damage until they can no longer see, speak or feed themselves.

First described in 1996, Variant Creutzfeldt-Jakob Disease (vCJD) is similar to Creutzfeldt-Jakob Disease as it is a transmissible spongiform encephalopathy, yet there are notable differences. First, young people are affected, with an average age of death under 30 years. Second, the disease has a relatively longer duration of illness. Finally, it is strongly linked to exposure, probably through food, to bovine spongiform encephalopathy (BSE). Other human transmissible spongiform encephalopathies have not been linked to food exposure.

Variant Creutzfeldt–Jakob disease (vCJD) is a type of [brain disease](https://en.wikipedia.org/wiki/Brain_disease) within the [transmissible spongiform encephalopathy](https://en.wikipedia.org/wiki/Transmissible_spongiform_encephalopathy) family (Ironside, 2012). Symptoms include [psychiatric problems](https://en.wikipedia.org/wiki/Psychiatric_problems), behavioral changes, and painful sensations. The length of time between exposure and the development of symptoms is unclear, but is believed to be years. Average [life expectancy](https://en.wikipedia.org/wiki/Life_expectancy) following the onset of symptoms is 13 months.

It is caused by [prions](https://en.wikipedia.org/wiki/Prion), which are mis-folded proteins. Spread is believed to be primarily due to eating [bovine spongiform encephalopathy](https://en.wikipedia.org/wiki/Bovine_spongiform_encephalopathy) (BSE)-infected beef. Infection is also believed to require a specific [genetic susceptibility](https://en.wikipedia.org/wiki/Genetic_susceptibility) (CDC 2015). Spread may potentially also occur via [blood products](https://en.wikipedia.org/wiki/Blood_products) or contaminated surgical equipment. Diagnosis is by [brain biopsy](https://en.wikipedia.org/wiki/Brain_biopsy) but can be suspected based on certain other criteria. It is different from [classic Creutzfeldt–Jakob disease](https://en.wikipedia.org/wiki/Creutzfeldt%E2%80%93Jakob_disease), though both are due to prions (Ferri and Fred, 2017).

[Variant Creutzfeldt–Jakob disease](https://en.wikipedia.org/wiki/Creutzfeldt%E2%80%93Jakob_disease) is a separate condition from [classic Creutzfeldt–Jakob disease](https://en.wikipedia.org/wiki/Classic_Creutzfeldt%E2%80%93Jakob_disease) (though both are caused by prions). Both classic and variant CJD are subtypes of Creutzfeld–Jakob disease. There are three main categories of CJD disease: sporadic CJD, hereditary CJD, and acquired CJD, with variant CJD being in the acquired group along with iatrogenic CJD (Geschwind, 2015).

International Statistical Classification of Diseases and Related Health Problems 10th Revision has no separate code for vCJD and such cases are reported under the Creutzfeldt–Jakob disease code (A81.0) (WHO 2016). The classic form includes sporadic and hereditary forms. Sporadic CJD is the most common type (CDC 2018).

There haven’t been any case of variant Creutzfeldt-Jakob disease recorded among blood donors in Nigeria due to the fact that screening for this transfusion transmissible infection is not carried out on donors and patients. This study is necessary for the close observation of blood donors in Nigeria, using Owo, a central city in Nigeria. Hence, there is need for surveillance study on donors for Variant Creutzfeldt-Jakob disease to forecast the possibility of transmitting same, and advocate a review of blood safety policy, as occasion may demand.

**AIM**

The study was carried out to determine the seroprevalence of Variant Creutzfeldt-Jakob disease prion among blood donors in Owo, southwestern Nigeria.

**MATERIALS AND METHODS**

**STUDY AREA**

The study was conducted at Federal Medical Center, Owo, Ondo state. The blood samples were tested for variant Creutzfeldt-Jakob disease. Study group include 90 blood donors who earlier tested negative for Human Immunodeficiency Virus (HIV), Hepatitis B surface Antigen (HBsAg) and Hepatitis C Virus (HCV).

**SAMPLE TECHNIQUE**

Convenience sampling was used to select blood donors in Federal Medical Centre Owo. The major reason for choosing convenience sampling is that donors were selected based on availability and willingness to take part, and there is no data on prevalence of this rare disease.

**SAMPLE COLLECTION**

Written informed consent was taken from each participant before the blood sample collection. Precautions were taken during blood collection. From each individual included in this study, 5ml of blood was drawn by vein puncture using disposable syringes. The blood was placed in plastic disposable plain bottle, it was left to stand at room temperature (20-25⁰C) to allow it to clot, and then the sera were separated by centrifugation for 5 minutes at 3000 rpm. The sera were stored, frozen at -20⁰C till analysis. All sera and reagents were allowed to stand at room temperature before use in the test. The samples were screened for Human Prion protein of variant Creutzfeldt-Jakob disease by PRNP ELISA kit.

**ETHICAL CONSIDERATIONS**

This study was subjected to ethical approval by Ethical and Research committee, Federal Medical centre Owo, Ondo state which was granted. Also informed consent was obtained from every participant.

**PRION PROTEIN ENZYME LINKED IMMUNOSORBENT ASSAY**

Enzyme linked immunosorbent assay (ELISA) kit produced for the quantitative determination of human major prion protein (PRNP) concentrations in serum, plasma, tissue homogenates by DIAPRO, Italy was used.

**Assay Procedure**

All the 96 wells were made used of (6 standard wells and 90 sample wells). 50μl of standard was added to standard wells. 40μl of sample was added to sample wells, 10μl of anti-PRNP antibody was added to sample wells, 50μl of streptavidin-HRP was added to sample wells and standard wells it was properly mix, the plate was covered with a sealer and incubated at 37°C for 60 minutes. The sealer was removed; the plate was washed 5 times with wash buffer. Wells were soaked with at least 0.35ml of wash buffer for 30seconds for each wash. 50μl of substrate solution A was added to each well and 50μl of substrate solution B was added to each well also. The plate was covered with new sealer and incubated at 37°C for 10 minutes. 50μl of stop solution was added to each well, the blue color changed to yellow immediately. The optical density of each well was determined immediately using a microplate reader set to 450 nm.

**STATISTICAL ANALYSIS OF DATA**

The results obtained in this study were analyzed statistically using a Statistical Package for Social Sciences (SPSS) version 20.

**RESULTS**

Out of the ninety blood donors screened for variant Creutzfeldt –Jakob disease prion ,none was found to be seropositive giving overall prevalence rate of 0%.

Table 1 shows that majority of the participant were females 74.4% with participant in age group 21-30 who were 40 in number being the majority (44.4%) in the age group categorization. The mean age and standard deviation were 31.244 and 22.117. A great number of the participants (52.2%) were unmarried

Table 2 shows that majority of participant (90%) have heard about (information) vCJD the major source of this information being from friends (38.9%), about a one-third of these participants gave virus as the vector (33.3%). A great number of participants (58.9%) put down weight loss as symptom. A total of 72 participants (80%) were aware of its prevention.

Table 3 shows that age group, gender and marital status does not have any significant effect on variant Creutzfeldt –Jakob disease prion, when subjected to person’s chi square test where all p values are higher than 0.05.

**Table 1: Socio-demographic variables of blood donors**

|  |  |  |
| --- | --- | --- |
| **Age group** | **Frequency** | **Percentage** |
| ≤20yrs | 6 | 6.7 |
| 21-30yrs | 40 | 44.4 |
| 31-40yrs | 33 | 33.3 |
| 41-50yrs  Total | 11  90 | 12.2  100.0 |
| **Sex** |  |  |
| Male | 23 | 25.6 |
| Female  Total | 67  90 | 74.4  100.0 |
| **Marital Status** |  |  |
| Married | 38 | 42.2 |
| Widowed | 2 | 2.2 |
| Single | 47 | 52.2 |
| Divorced | 3 | 3.3 |
| Total | 90 | 100.0 |

Note

Mean age= 31.244

Standard deviation= 22.117

**Table 2: Knowledge and attitude about variant Creutfeldtz-Jakob disease**

|  |  |  |
| --- | --- | --- |
|  |  |  |
| **Information** | **Frequency** | **Percentage** |
| Yes | 81 | 90.0 |
| No  **Total** | 9  90 | 10.0  100.0 |
| **Sources** |  |  |
| Family | 2 | 2.2 |
| Friends | 35 | 38.9 |
| Media | 14 | 15.6 |
| School | 2 | 2.2 |
| Health workers | 26 | 28.9 |
| No specific place  **Total** | 11  90 | 12.2  100.0 |
| **Vector** |  |  |
| Virus | 30 | 33.3 |
| Bacterial | 23 | 25.0 |
| Parasite | 11 | 12.2 |
| Hot climate | 5 | 5.6 |
| Cold air | 7 | 7.8 |
| None | 9 | 10.0 |
| Cow | 5 | 5.6 |
| **Total** | **90** | **100.0** |
| **Symptoms** |  |  |
| None | 3 | 3.3 |
| Red eye spot | 9 | 10.0 |
| Weight loss | 53 | 58.9 |
| Rashes | 11 | 12.2 |
| Fever | 9 | 10.0 |
| Tiredness | 1 | 1.1 |
| Loss of appetite | 2 | 2.2 |
| Dementia | 1 | 1.1 |
| Hallucination | 1 | 1.1 |
| Total | 90 | 100.0 |

|  |  |  |
| --- | --- | --- |
| **Prevention** |  |  |
| Yes | 72 | 80.0 |
| No | 3 | 3.3 |
| I don’t know  **Total** | 15  90 | 16.7  100.0 |
| **Treatment type** |  |  |
| None | 23 | 25.6 |
| Drug | 57 | 63.3 |
| Vaccination | 4 | 4.4 |
| Herbs | 6 | 6.7 |

**Total** 90100.0

|  |  |  |
| --- | --- | --- |
|  |  |  |

**Table 3: Effect of socio-demographics variables on variant Creutfeldtz-Jakob disease** **prion**

|  |  |  |
| --- | --- | --- |
|  | p-value | Comment |
| Age group | 0.356 | Not significant |
| Sex | 0.699 | Not significant |
| Marital status | 0.304 | Not significant |
|  |  |  |

**DISCUSSION**

In this study, we investigated the prevalence of variant Creutzfeldt –Jakob disease prion among blood donors in owo. We also determined the risk of transmitting Variant Creutzfeldt-Jakob disease to blood unit recipients in Owo. The prevalence of Variant Creutzfeldt –Jakob disease prion among donors in Owo has 0% rate, which means there was no positive case recorded throughout the course of study.

Defect in PRNP are the cause of vCJD once there is defect in PRNP it triggers the normal protein in the brain to fold abnormally. Most reported vCJD cases appear to have been infected through the consumption of cattle products contaminated with the agent of BSE (Collee *et al*., 2006). In three cases, reported in the UK, the mode of transmission is thought to have been through receipt of a blood transfusion derived from an asymptomatic, but infected donor (Hewitt *et al*., 2006).

During infection with prion diseases, infectious titres of prion protein are present in peripheral tissues (particularly lymphoid organs and spleen) before a progressive rise in brain titres finally results in clinical disease. Subclinical or carrier states may have major public health implications for public health, particularly regarding potential iatrogenic transmission from apparently healthy persons (Smith *et al*., 2004). Variant Creutzfeldt –Jakob disease is characterized by progressive dementia and myoclonic seizures affecting adult in mid-life. Some patients present sleep disorders, abnormalities of high cortical function, cerebella and corticospinal disturbances. The disease ends in death after a 3-12 months illness.

Preventive measure of vCJD has not been laid down yet in Nigeria ma ybe due to the fact that no case has been recorded yet; several control and prevention measures have been implemented in the Europe. This include feed ban, which is the basic preventive measure laid down and consists of a ban on the use of processed animal protein (PAP) in feed for farmed animals. Based on scientific findings that linked the spread of the disease to the consumption of contaminated PAP feed, a ban on the feeding of mammalian processed animal protein to cattle, sheep and goats was introduced since the UK in 1988 and in other member states in July 1994. Aiming at eradication of certain TSE, the ban was expanded in January 2001 with the feeding of all processed animal proteins to all farmed animals being prohibited, with certain limited exceptions. In 2009 a further ban on the use of milk and milk products coming from classical scrapie infected flocks for feeding ruminants was set out (Chronological legislation, 2013). As a result, the number of affected cattle in the UK declined steadily, with only 3 reported cases in 2013 (Annual reports of member states, 2013).

In the United Kingdom vCJD infection was observed in three recipients of blood transfusions from two donors who later developed the disease and one blood recipient who died of another cause without clinical symptoms, but who at autopsy had prions in spleen and lymph nodes. The possibility of a risk has been assessed for plasma products, human organ and tissue transplants and contaminated surgical instruments or devices (Collee *et al.,* 2006).

**CONCLUSION**

This study found that there is no prevalence of variant Creutfeldt-Jakob disease among blood donors in owo. Including screening for variant Creutzfeldt –Jakob disease in the blood safety policy of Nigeria is not advised, as of now. Surveillance should be sustained to review the policy, as entrenched in national blood transfusion safety programmes

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