Safety of Equine Tetanus Antitoxin for Prophylactic Use in Ethiopia: A Retrospective Multicenter Study

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A thesis submitted to CDT-Africa, College of health sciences, Addis Ababa University on the partial fulfillment of the requirements for the Degree of Masters of Science in clinical trials.

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Declaration

This thesis is my original work and has not been presented for a degree in any other university, and that all sources of material used for this thesis have been properly recognized.

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This is to certify that thesis prepared by **Michele Joseph**, titled "**Safety of Equine Tetanus Antitoxin for Prophylactic Use in Ethiopia: A Retrospective Multicenter Study** (**TAT-Safe),** has been submitted in partial fulfillment of the requirements for the degree of Master's of Science in Clinical Trials, complies with the regulation of the university and meets the accepted standards with respect to originality and quality.

Examining Board

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i. Abbreviations/Acronyms

AEFI	Adverse Event Following Immunization
CDT-Africa	Center for Innovative Drug Development and Therapeutic Trials for Africa
EFDA	Ethiopian Food and Drug Authority
FMoH	Federal Ministry of Health
HIV	Human Immunodeficiency Virus
IU	International Unit
PEP	Post Exposure Prophylaxis
PFSA	Pharmaceutical Fund Supply Agency
TAT	Tetanus Antitoxin
TIG	Tetanus Immunoglobulin
TT	Tetanus Toxoid
WHO	World Health Organization

ii. Acknowledgment

First, thanks to God, the Almighty for his blessings throughout my works.

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I am extremely grateful for all the staffs of CDT- Africa who were all there for me for the past two years, this would not have come true without you.

iii. Abstract

Background: Tetanus remains an important public health problem in many parts of the world. It is indicated to cause an estimated 56,000 deaths annually worldwide. Among global tetanus deaths, 44% occur in sub-Saharan Africa, and the highest proportion of these occurs in East Africa. In 2017 WHO reported 20 cases of tetanus in Ethiopia. Although tetanus antitoxin (TAT, equine origin) has been replaced by human tetanus immunoglobulin (TIG), it is still in use in developing countries due to the high costs and limited access to TIG. Adverse reactions of TAT have not been studied in Ethiopians. Thus, conducting a Retrospective safety study on TAT in Ethiopia is important to promote public health and support improvement of the product by providing scientific evidence from the local context.

Objective: To evaluate the safety of equine tetanus antitoxin when administered to adults under conditions of routine post-exposure prophylactic use in Ethiopia.

Methods: A retrospective multicenter study was conducted in Addis Ababa covering three referral teaching public hospitals, (Tikur Anbessa Specialized Teaching Hospital and Zewditu Memorial Teaching Hospital and) and eight public health centers of with high client load for TAT. Charts of patients (n=1213) treated with equine anti toxin for prophylactic use for their wounds from January 2015 to December 2019, older than 18 years of age were reviewed.

Results: We included 1213 medical record forms in the final analysis (994 males and 264 females) and the mean age was 29.33 years (SD = 11.41 years). Only 0.98%(12/264) of the females had history of TT vaccination. Among all the charts reviewed head trauma, trauma at workplace and open wound were most frequently seen and only one patient experienced adverse event following immunization.

Conclusion and Recommendation: Even though there were major limitation on the data retrieved, on its clarity and completeness, findings from this retrospective study suggests that use of TAT is safe compared to number of injections and the overall disease reported in the country from January 2015 to December 2019. Large study with wider geographical converge will have significance in testing the validity of the current findings.

Key words: equine tetanus anti-toxin, tetanus immunoglobulin, safety

1. Background

1.1 Description

Tetanus is a bacterial infection that affects the central nervous system. The causative agent, *Clostridium tetani*, is a spore forming, toxin-producing gram-positive, anaerobic bacillus found in the environment irrespective of geographical location [1, 2]. The spores are extremely resistant to heat and to the conventional antiseptics, being able to survive for many years in adverse conditions. Tetanus may follow puncture wounds, surgery, burns, crush wounds, otitis media, animal bites, oral infections, or childbirth and abortion. The spores enter the body through these routes and produce the toxins tetanolysin and tetanospasmin in anaerobic conditions [3, 4]. The toxin migrates to the central nervous system through retrograde axonal transport within nerve cells and causes muscular rigidity and spasms [5]. Incubation period is usually 8–10 days (range 3–21 days) following infection of a wound. Among patients in the youngest and oldest age groups, the case-fatality rate approaches 100% without intensive care [6]. Diagnosis is entirely based on clinical features and does not depend on laboratory confirmation, for *C. tetani* is recovered from microbiologic culture of wounds in only about 30% of cases [6, 7]. The combination of a history of injury with a tetanus-prone wound and muscular spasms could increase the possibility of the investigation [8]. Wound management, a single intramuscular dose of human or equine tetanus antitoxin, and antibiotics prevent further progression of the disease [7].

1.2 Epidemiology

Epidemiologically, tetanus remains an important public health problem in many parts of the world where immunization programs are suboptimal. It causes an estimated 56,000 deaths annually worldwide, approximately 20,000 of them are neonates [3], while in many countries, tetanus disease surveillance is not well established, and thus its incidence is not accurately known. Among global tetanus deaths, 44% occur in sub-Saharan Africa and the highest proportion is in East Africa [9]. In Africa, there were 2,791 and 5,787 reported cases of Tetanus in 2017 and 2018, respectively [10]. In Ethiopia, there were 20 reported cases of tetanus in 2017 as per the WHO report [10].

1.3 Prevention

Prophylaxis of tetanus consists of local wound management, active immunization, and passive immunization. An active immunization, a six-dose series of tetanus toxoid vaccine, protects by

stimulating the production of antitoxin, providing immunity against the effects of the toxin [3]. A passive immunization, which could be tetanus immunoglobulin (TIG) (human) or tetanus antitoxin (TAT, equine origin), is recommended as post-exposure-prophylaxis within 24 hours after a tetanus prone wound has occurred. Both TIG and TAT are currently available in the global market. The antitoxins neutralize any circulating toxin before it reaches the nervous system [11], but cannot remove the toxin that is bound to nerve endings [8]. Due to the strong effect of its toxins, tetanus infection does not produce tetanus immunity [8].

1.4 Equine tetanus antitoxin

Equine tetanus antitoxin (TAT) is made of toxin-neutralizing immunoglobulin fragments F (ab')2, extracted, and purified from tetanus toxoid-immunized horse blood [12]. For children and adults, it is administered in 1500 IU single dose or 3000 IU if more than 24 hours has elapsed [13]. It is administered following Besredka's method by injecting 0.1 ml subcutaneously and waiting for 15 minutes; if no local or general allergic reactions occur, 0.25 ml is injected subcutaneously, and observed for 15 minutes; if no reactions, it is given by IM route [13]. The half-life of TAT in adults is 1-2 weeks [36, 39]. In persons who have not had horse serum previously, the antitoxin reaches very high levels in the serum during the first week after injection, but the human host treats it as foreign protein and its level drops sharply after the 10^{th} day.

It has been estimated that 5-6% of adult patients receiving TAT had some adverse reaction [14], while it is not contraindicated in case of pregnancy or breast-feeding [13]. Anaphylactic shock may occur despite a negative sensitivity test or may result from the test dose itself [15]. It may cause hypersensitivity reactions, such as anaphylactic shock, Quinke oedema, or serum sickness up to 10 days after injection [13]. It should not, therefore, be administered to patients with known allergy to tetanus antiserum.

TAT was used ever since the first world war (15) but, Human TIG was developed in 1960. following the adverse effects of the equine tetanus antitoxin [11]. Human TIG is more efficacious and long lasting than TAT [16]. TIG persists for much longer in serum (4-5 weeks) than does TAT and seems to have fewer adverse reactions [14]. Due to its human origin, TIG can be applied directly without skin test, unlike TAT; however, since it is a human blood product, potential risk of infections with human viruses, such as viral hepatitis, HIV/AIDS, and other infectious diseases, still remain to be a problem [17]. In most developed countries, TAT is totally replaced by TIG,

while TAT products are still in use in developing countries due to the high costs and limited access to human TIG [18].

The subject of this study which is equine tetanus antitoxin which is produced and manufactured by the ViNS Bioproducts Limited, India (Code: 130202084, A.W.No: 15/AAW/PI/02.00, DT: 25.04.2016). The product is licensed and in use in Ethiopia since 2015. It is prepared by hyper immunizing horses with tetanus toxoid. Plasma obtained from healthy immunized horses is enzyme refined, purified and concentrated. The tetanus antitoxin has the specific antitoxic immunoglobulins which neutralize the toxin formed by *C tetani*. The product is supplied as 1 ml liquid in a glass vial/ ampoule and also supplied as freeze-dried powder with 1 ml of water for injection I.P. for reconstitution. Each mL contains: enzyme refined, equine tetanus antitoxic immunoglobulin fragments not less than 1500 IU, cresol BP < 0.25% v/v as a preservative, glycine as a stabilizer BP, sodium Chloride BP, and water for Injections BP.

The product is delivered with the dose of 1000/1500 IU intramuscularly or subcutaneously to individuals at risk of tetanus infection for prophylactic purposes. The dose may be doubled or tripled in case of multiple and severe wounds. Prophylactic dose is also given in surgical operations as post-operative care. Prior to receiving the product, the individual needs to be tested for hypersensitivity, which is carried out by injecting 0.1 ml tetanus antitoxin serum in 1:10 dilution either subcutaneously or intradermally and observing for any local or general reaction for half an hour.

Injection of the antitoxin to individuals with a history of allergic reactions to equine protein and to individuals with asthma, infantile eczema is contraindicated. The manufacturer advises that adrenaline injection (1:1000) must be available for immediate treatment of shock if it develops. The manufacturer's leaflet is attached with this proposal as annex 1.

1.5 Study rationale

In Ethiopia, recent studies have reported a high case-fatality rate of patients admitted to hospitals due to tetanus infection [19,20,21] and a low history of tetanus toxoid vaccination [21, 22, 23]. According to the Ethiopian Federal Ministry of Health [FMoH] healthcare facilities' capacity assessment held in 2016, 42% (1598/ 3,804) of healthcare facilities lack TAT, though 89% of them have tetanus toxoid vaccine [24]. The FMoH standard treatment guideline recommends consideration of tetanus and rabies prophylaxis for all wounds [25]. The healthcare provider needs to determine the patient's tetanus immune status; if status is inadequate or unknown, the provider should administer Human TIG or TAT with close follow up of the potential adverse events following immunization.

The Ethiopian Food Medicine and Healthcare Administration and Control Authority (FM-HACA), the former national regulatory body of Ethiopia licensed an equine tetanus antitoxin in 2015. Equine tetanus anti-toxin is manufactured by the ViNS Bioproducts Limited, India (Code: 130202084, A.W.No: 15/AAW/PI/02.00, DT: 25.04.2016) for marketing and it is in use in Ethiopia as a post exposure prophylaxis (PEP). The Pharmaceuticals Fund and Supply Agency **(**PFSA) of Ethiopia has included the product in the 2018 national pharmaceuticals procurement list (Serial No. 318, Base Code: Teta-30, Description: Tetanus Antitoxin (TAT), Equine - 1,500 IU/ml in 1ml Ampoule – Injection, Unite: 20) [26]. FM HACA renamed currently "Ethiopian Food and Drug Authority (EFDA)" procures and distributes the product to public healthcare facilities for prophylactic use against tetanus.

For equine driven TAT, though there have been no formal studies conducted in Ethiopia, evidences are available that they can cause anaphylaxis [27], late serum sickness [27], and high toxicity [28], while these products are still in use due to their low costs [17, 29]. Immediate systemic allergic reactions after administration of this product have not been documented in Ethiopia.

Thus, conducting retrospective safety study on the TAT product manufactured by ViNS Bioproducts Ltd is important to evaluate the safety of equine tetanus antitoxin, promote public health and to support improvement of the product by providing scientific evidence from a local context.

2 Aim

The aim of this retrospective cohort study was to evaluate the safety of equine tetanus antitoxin, (Code: 130202084, A.W.No: 15/AAW/PI/02.00, DT: 25.04.2016, VINS Bioproducts Limited, India) when administered to adults under conditions of routine post-exposure prophylactic use in Ethiopia.

3 Methods

3.1 Study design

The study was multi-site retrospective cohort study.

3.2 Setting

The study was conducted in healthcare facilities in Addis Ababa, Ethiopia; three referral teaching public hospitals, (Tikur Anbessa Specialized Teaching Hospital and Zewditu Memorial Teaching Hospital) and eight Public health centers (Arada Health Center, Churchill Health Center, Afinchober Health Center, Yeka Health Center, Kirkos Health Center and Nifas Silk Lafto W/9 Health Center) with high client load for TAT. To facilitate the selection process of health facilities, investigators reviewed documents at Addis Ababa Health Bureau and Ethiopian Pharmaceutical Supply Agency. This review helped to identify health centers with high TAT clients load.

3.3. Context

The Ethiopian health system has three tire health structure (health centers, primary hospitals and general hospitals), when going up through the system that is applicable for the urban population, larger population are covered. Primary hospitals and general hospitals serve as referral center for health centers and primary hospitals respectively [30]. During the referral system appropriate record keeping is mandatory as maintaining a high quality clinical record facilitates greater communication among physicians, treatment continuity and policy making [31]. Incomplete/illegible records obstruct the patient's care as well as policies based on the records., excellent record keeping is a standard for evidence based decision making. [32].

3.3 Source population and study participants

The source population was all adults who visited emergency departments of the three hospital and the eight health centers seeking medical service for their trauma. The study participants were all adults of at least 18 years who were treated with equine anti toxin for prophylactic use for their wounds since January 2015 to December 2019. Charts which were unclear/unreadable/not properly recorded were excluded and also documented and reported as an input for future quality improvement purposes

3.4 Sample size

We reviewed all charts of patients who were treated in the study health facilities for trauma and related issues since January 2015 G.C to December 2019 G.C. Considering occurrence of any adverse event following immunization (AEFI), a sample size of 601 was enough to estimate the population parameter with the following assumed inputs: (a) 6% incidence of AEFI with 2%

margin of error, (b) 95% confidence in the estimate, (c) 10% non-response rate including incomplete records.

The sample size was determined by using single proportion formula

$$n = \frac{(Z_{1-\alpha})^2 \times P\,(1-P)}{\delta^2}$$

$$n = \frac{(1.96)^2\,(0.06)\,(0.94)}{(0.02)^2} = 601$$

But during the data collection we managed to include charts of 1213 patient's charts.

3.5 Sampling Procedure

Charts of patients who visited departments of emergency, outpatient and surgery of the eight health facilities were included after reviewing the logs of these departments. Registration logs were reviewed only for isolating eligible charts of patients, then charts of eligible patients were separated from the other cards and reviewed.

3.6 Definition of terms

In this study, the safety of the TAT product was evaluated and interpreted according to the WHO definition for adverse events following immunization (AEFI) and serious AEFI [33]. The study data collection tool (Annexure 4) included parameters that indicated AEFI and Serious AEFI. The decision for inclusion as AEFI or Serious AEFI was based on the data recorded by the physicians.

3.6.1. Adverse events following immunization

The WHO defines Adverse Events Following Immunization (AEFI) as any untoward medical occurrence following immunization and which does not necessarily have a causal relationship with the usage of the vaccine [33]. The adverse event may be any unfavorable or unintended sign, abnormal laboratory finding, symptom or disease. Reported adverse events can either be true adverse events, i.e. resulting from the vaccine or immunization process - or coincidental events that are not due to the vaccine or immunization process but are temporally associated with immunization [33].

3.6.2. Serious adverse events following immunization

Serious adverse events following immunization (Serious AEFI) is an AEFI which results in death, is life-threatening, requires in-patient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, or requires intervention to prevent permanent impairment or damage [33]. "Severe" is used to describe the intensity of a specific event (as in mild, moderate or severe).

3.7 Procedures

We collected data relevant to analysis of the safety of the TAT. The process of data collection included reviewing charts using structured, standardized and pretested checklist.

3.8 Outcome measures

3.8.1 Primary outcome measures

- Number of participants with Serious AEFI due to the TAT [Time frame: within 21 days of receipt of the TAT].
    - Number of participants experiencing Serious AEFI related to the TAT, occurring within two hours of administration of the TAT, measured as observed by study clinician or reported by the participant to study clinician,

3.8.2 Secondary outcome measures

- Number of Participants with solicited AEFI [Time Frame: from two hours after administration of TAT through 21st day]
    - Number of participants experiencing AEFI related to the TAT, commonly associated with local and systemic reactions, occurring greater than two hours after administration, measured as observed by study clinician or reported by the participant to study clinician.

3.8.2. Main outcome

AEFI

3.8.3 Independent Variables

- Demographic
  - Age
  - Sex
  - Residence
  - Occupation

- Health status
  - General health condition
  - Weight
  - Height
  - HIV test
  - CD4 count

- Nature of injury
  - Place of injury: home; school; street; others
  - Activity: work; education; sport; traveling; others
  - Mechanism: traffic; sexual assault; fall; blunt force; stab/cut; gunshot; fire/heat
  - Nature: fracture; sprain/strain; cut/bite; open wound; bruise; organs system injury
  - Site of injury: specific body part/s injured
  - Time of injury: estimated time from injury onset to clinical visit

- Vaccination history
  - Status of previous history of TT vaccination

3.9. Ethical consideration

The study was reviewed and approved by: I) the Institutional Review Board of the College of Health Sciences, Addis Ababa University (protocol No 071/19/CDT), and II) the Addis Ababa City Administration Health Bureau. All study sites gave permission in writings for data collection.

All the information retrieved from charts review and information about the study sites were identified by code number to protect confidentiality of participants. All records were kept locked at CDT-Africa office. All computer entry and networking programs were locked with password. Participant's information will not be released to third party without written permission of the participant. Only the investigators had access to the row data.

3.10 Data analysis

Data was entered to EpiData version 3.1 and then transported to STATA version 14, AEFI of TAT and baseline demographics of participants were summarized descriptively using proportions (when they were categorical) and means (when they were continuous). Baseline characteristics and nature of wounds were summarized in tables and figures.

3.11 Quality assurance and study monitoring

Study staff received a formal training on the data collection tools before data collection started, and the study investigators actively monitored and were engaged in the data collection process. Supervisions were done by means of on-site visits to the facilities and through other communication methods such as telephone calls or written correspondence. The visits were scheduled at mutually agreeable times, and the frequency of visits was at the discretion of CDT Africa. During the visit, any study-related materials were reviewed

4. Results

More than twenty thousand patients were treated for trauma in the facilities from January 2015 G.C to December 2019 G.C (annual reports from health facilities). However, most of the health facilities destroyed their registration books to empty their limited space, negligence, or while moving departments and cleaning to in response to the current pandemic. We were only able to retrieve registration books for a maximum of two years', anywhere between 2015 and 2019. Moreover, the retrieved records were archived in unsuitable places in inappropriate condition.

The registration books that were made available were reviewed. Upon revision of these registration books, 6000 charts were eligible for the study. Two hundred charts were excluded from the study because of missing or unreadable Medical Record Numbers, 98 patient charts were excluded from the study because they were referred from other health facilities after being given TAT and were

not useful to precisely evaluate the safety profile of the vaccine. The remaining 5702 charts were eligible for the study and 1500 of these charts were included in the study as it was noted that they were given TAT for their trauma at the health facilities. Among the 1500 charts 287 charts were excluded because it was not possible to confirm from registration logs of the health facilities whether they took the vaccine or not. Hence, a total of 1213 charts were included in the study for analysis (this flow is presented on figure 1).

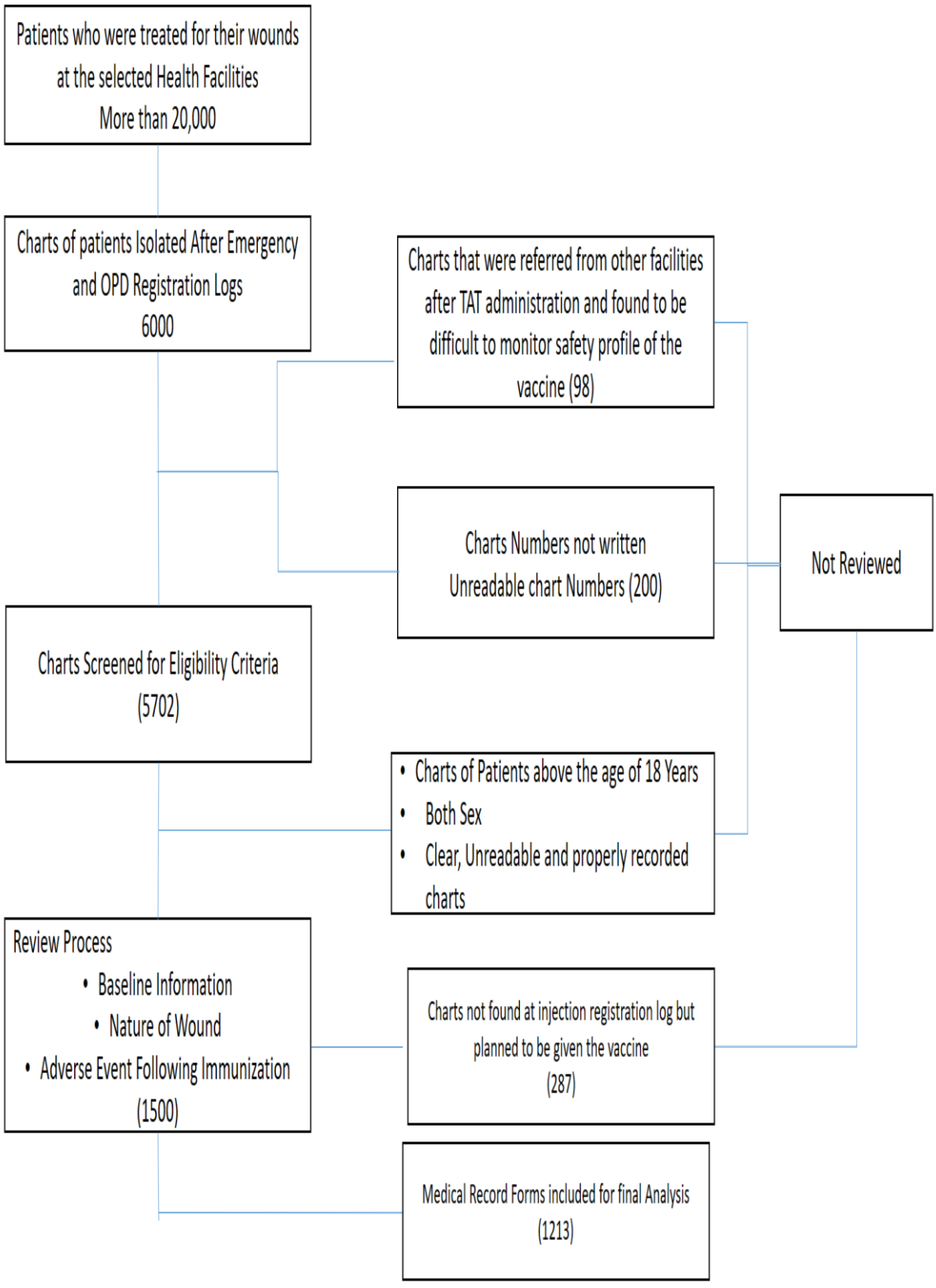

Figure 1. Flow chart that shows identification of charts eligible for review

4.1. Baseline Characteristics

A total of 1213 charts of patients were reviewed., except for the 2 health facilities where it was not possible to trace adverse events which occurred following immunization from the available registration logs. Male to female ratio of the study participates was 3.6:1, the mean age was 29.33 years (SD = 11.59 years) and the age of individual study participant ranges from 18 years to 91 years (table 1). The modal age lays with 18- 27 years and the age distribution was positively skewed (figure 2)

Table 1: Age of participants in category

Age category	**Sex**		**Total**
	Male	**Female**	
18-27	525	147	676
28-37	267	64	331
38-47	87	31	118
48-57	32	17	49
58-67	21	4	25
68-77	13	1	14
78-87	3	0	3
88-97	1	0	1
	949	264	1213

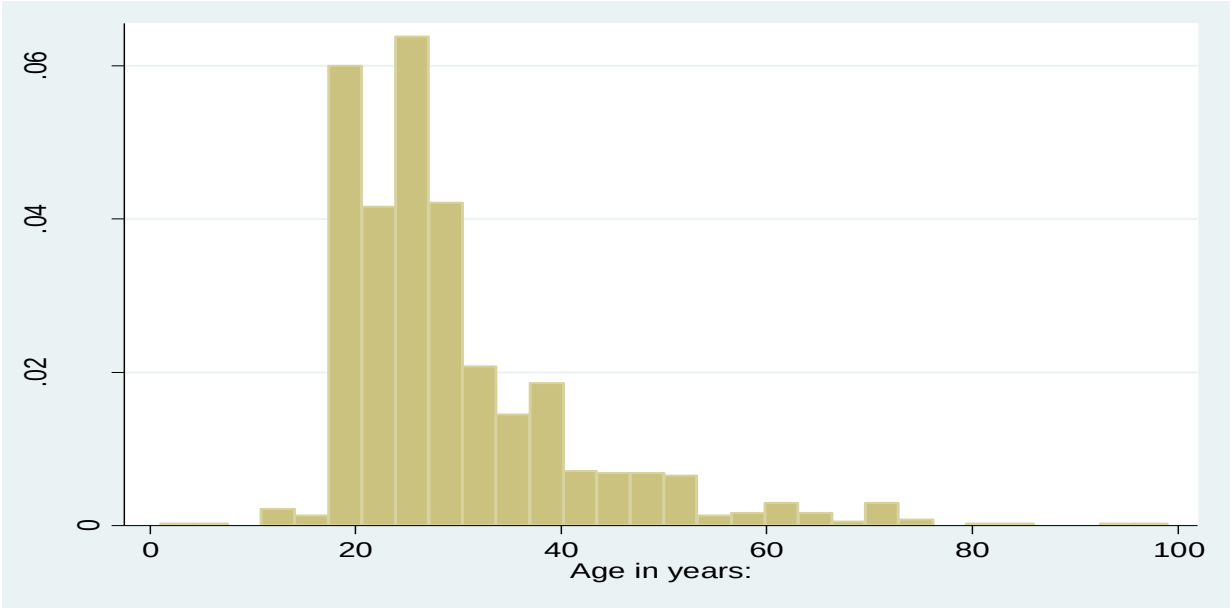

Figure 2: Histogram of age distribution of study participants.

Table 2. Distribution of baseline characteristics

Sex	Number of study participants	Percent
Male	949	78.23
Female	264	21.77
Residence		
Urban	1154	95.13
Rural	59	4.86
Literacy		
Literate	782	64.46
Illiterate	21	1.74
Unknown	410	33.80
Occupation		
No job	10	0.82
Student	12	0.99
House wife	4	0.33
Trader	1	0.08
Farmer	17	1.40
Government employee	2	0.16
Daily laborer	4	0.33
Other	16	1.32
Unknown	1147	94.57
Marital status		
Never married	16	96.46
Married	25	2.06
Widower	2	0.16
Unknown	1170	96.46
Educational status		
None	15	1.24
Elementary	12	0.99
Secondary	12	0.99
Preparatory	7	0.58
University (diploma)	3	0.24
University (degree)	1	0.08
University (MSc., PhD)	1	0.08
Unknown	1162	95.80

Most of the participants (n=1154, 95.13%) were from urban areas and 64.46% were literate (table 2)

The HIV status of almost all study participant was not known, and few (n=21, 1.73%) charts were identified to have TT vaccination history. All of the 12 study participants those whose vaccination history was known were females in the age range of 18-37 years (tables 3-4).

Table 3. TT vaccination and HIV history

TT Vaccination History	n	%
Yes	12	0.99
No	9	0.74
Unknown	1192	98.27
Total	1213	100
HIV History		
Positive	2	0.16
Negative	75	6.18
Unknown	1136	93.66
Total	1213	100

4.2. Nature of Wounds

Most of the reported cases happened while they were on the street (n= 522, 43.03%) and most 19.78% suffered from the trauma when they were at their workplace. Almost all of the charts did not state the type of work they were doing while the trauma happened, but workshops and machinery works were among the stated ones. Open wound (n=930, 76.66%) was among the most frequently occurring type of wound. Stab/cut (n=535, 44.10%) and blunt force (n=362, 29.84) were the most frequently reported mechanisms by which TAT vulnerable wounds occurred (figure 3-4). The mean time of presenting at health facilities from the onset of trauma was 2.96 hours. The earliest and late time of presenting at health facilities were 0.083 hours (5 minutes) and 30 days, respectively. The modal time of presenting at health facilities was 15 to 30 minutes. (figure 7) Those people coming from school, working and suffered open wound and traffic accident were the ones who came earlier to hospitals. Heads (n=253, 23.03%) and Hands (n= 270, 22.78 %) were

very common site of injuries. Activities, nature and mechanism of occurrence of wound are presented on table 5.

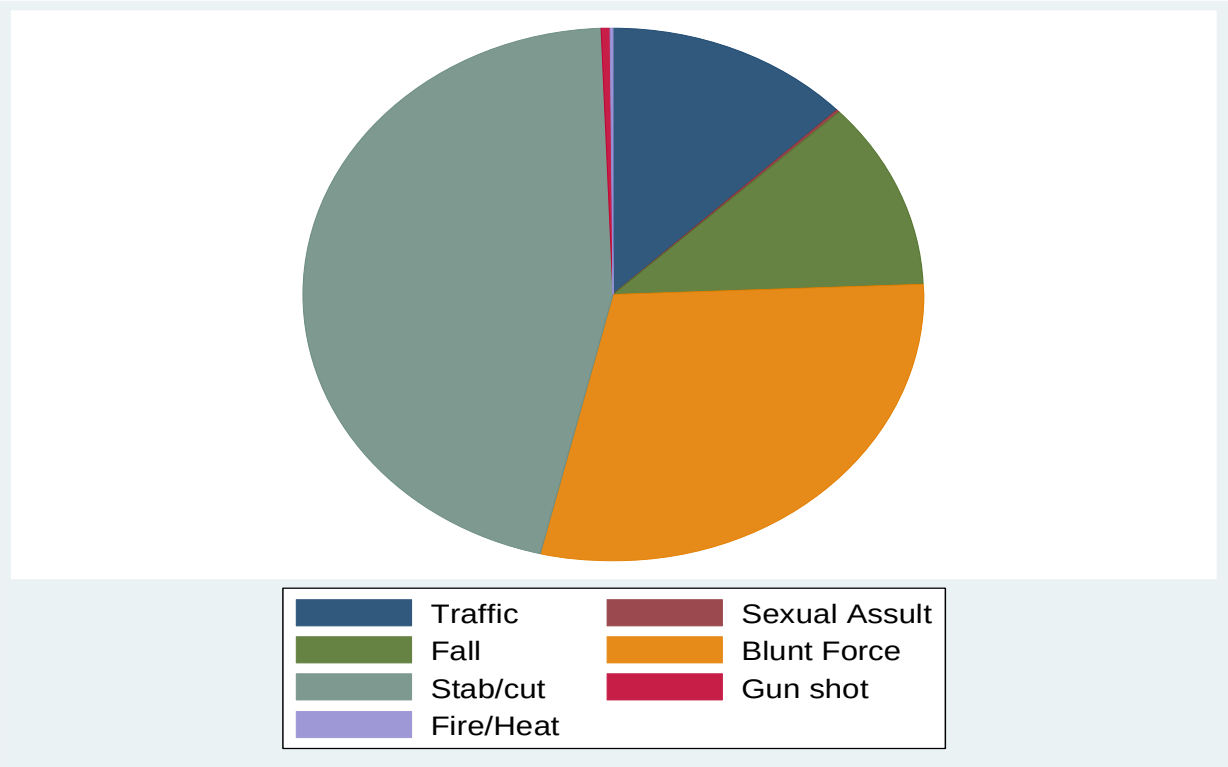

Figure 3: Pie chart showing mechanism of trauma

Table 4. Activities, nature and mechanism of occurrence of wound

	n	%
Activities		
Work	240	19.79
Education	2	0.16
Sport	26	2.14
Travelling	229	18.88
Unknown	386	31.82
Other	330	27.21
Nature		
Open wound	930	63.48
Fracture	107	7.30
Sprain/strain	25	1.71

Cut/bite	296	20.21
Bruise	103	7.03
Organ system injury	4	0.27
Mechanism		
Traffic	169	13.93
Assault	2	0.16
Fall	133	10.96
Blunt force	364	30.01
Stab/cut	536	44.20
Gunshot	7	0.58
Fire/heat	2	0.16

N.B. A person may experience more than one type of wound (nature)

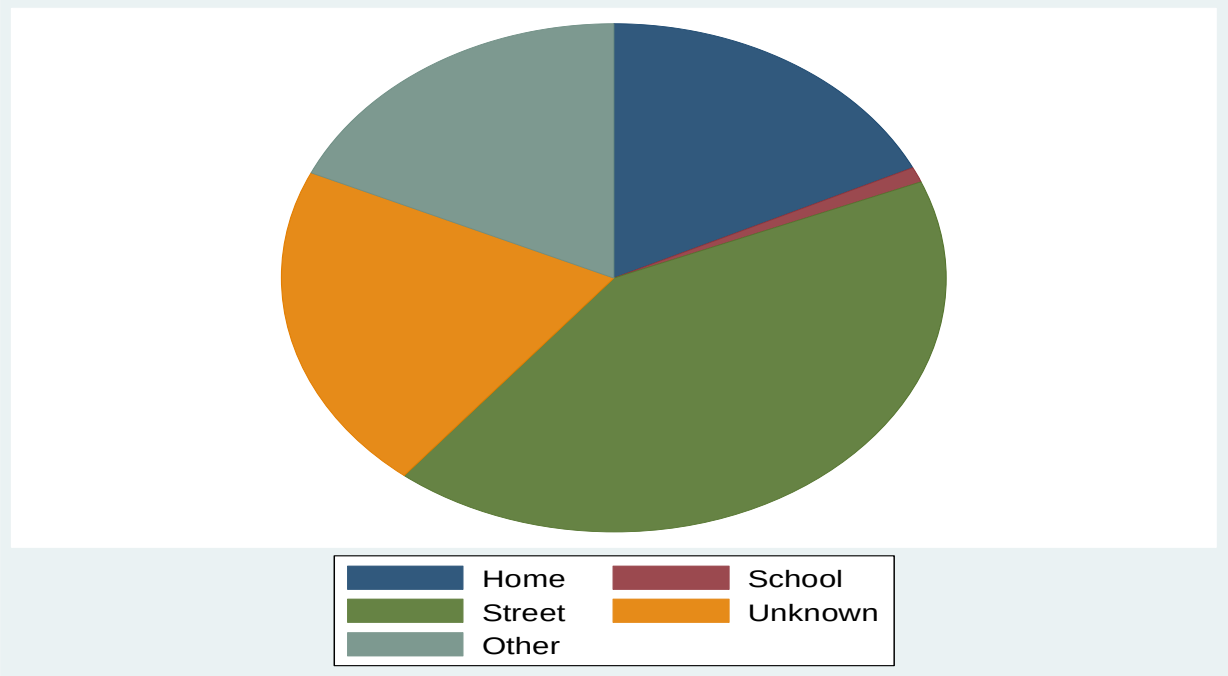

Figure 4: Pie chart showing place where the case occurred

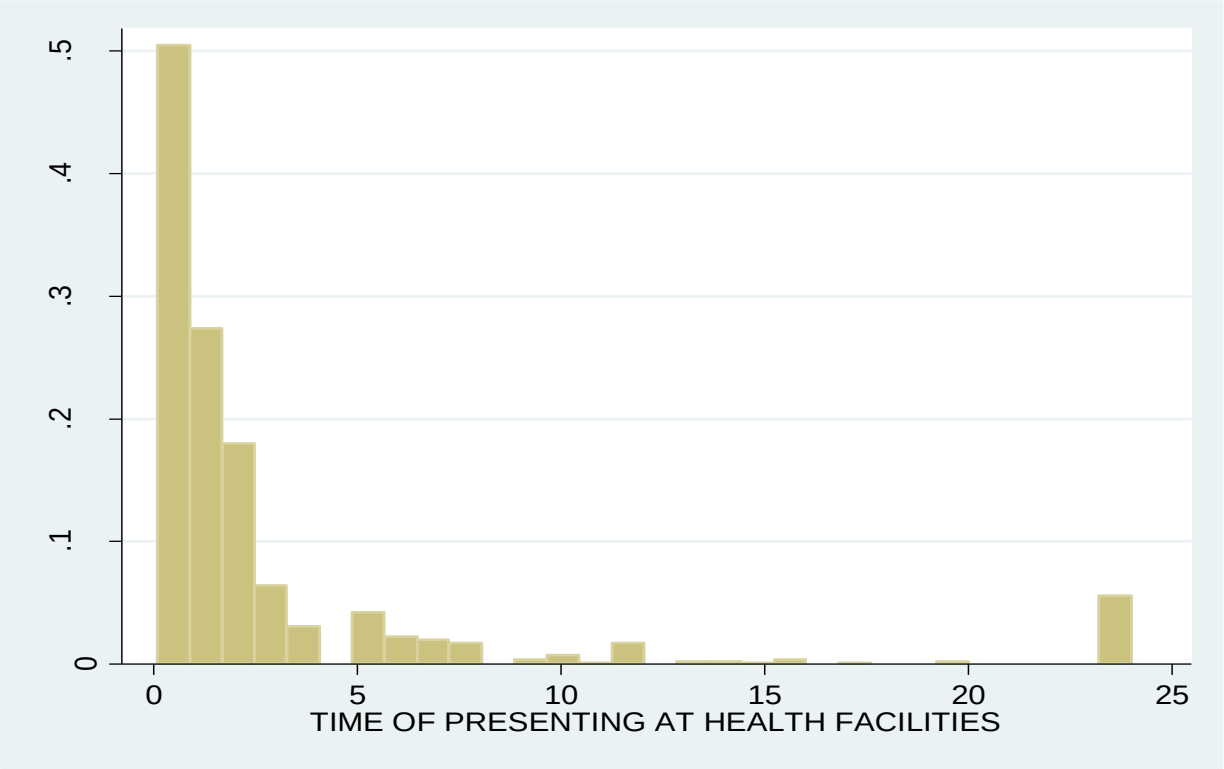

Figure 7: The histogram for the distribution of time of presenting at the health facilities from onset of trauma

Most of the patients come to health facilities within the first few hours after experiencing trauma (figure 7).

4.3. Adverse Event Following Immunization

Since the patients included in the study were above 18 years of age, the majority (n=1139, 93.89%) were given 3000 IU, and only three patient received 1500 IU. From all the charts included in the study, 10.96%, 2.55% and 5.85% did not indicate whether skin test was performed before giving the drug, the route of administration, or none of the information that was expected to be provided even including the dosage, respectively. Only the dosage to be given to the patient, and no other information required was indicated in most of the charts (n=834, 71.22%). One hundred forty patient's charts (11.87%) had whole information like the dosage, route of administration, and prior skin tests to be performed. Even though 6 (0.49%) patients returned or stayed at the hospital on or until the 21st day, none of the visits had a relation with administration of TAT.

One male patient aged 23 years presented within 3 hours to Zewditu memorial hospital after experiencing trauma on his nose while he was at his work place. His HIV status and TT vaccination histories were unknown. He was given TAT 3000 IU intramuscularly soon after he was admitted,

he experienced severe local reaction immediately after injection and it was confirmed that the reaction was related to the TAT given, by the physician in charge. However, no record was found on the Medical Record Form regarding what measures were taken following the reactions, and the patient did not show up at the 21st day after injection.

Figure 6 shows the flow chart that health facilities currently practice for emergency patients when a patient visits emergency ward, they will be sent to injection room without their medical record form accompanying them to the injection room, if they need any kind of injection including TAT, and if the patient experiences either a positive skin test or mild adverse event there is no mechanism to report it to the physician in charge (current practice observed).

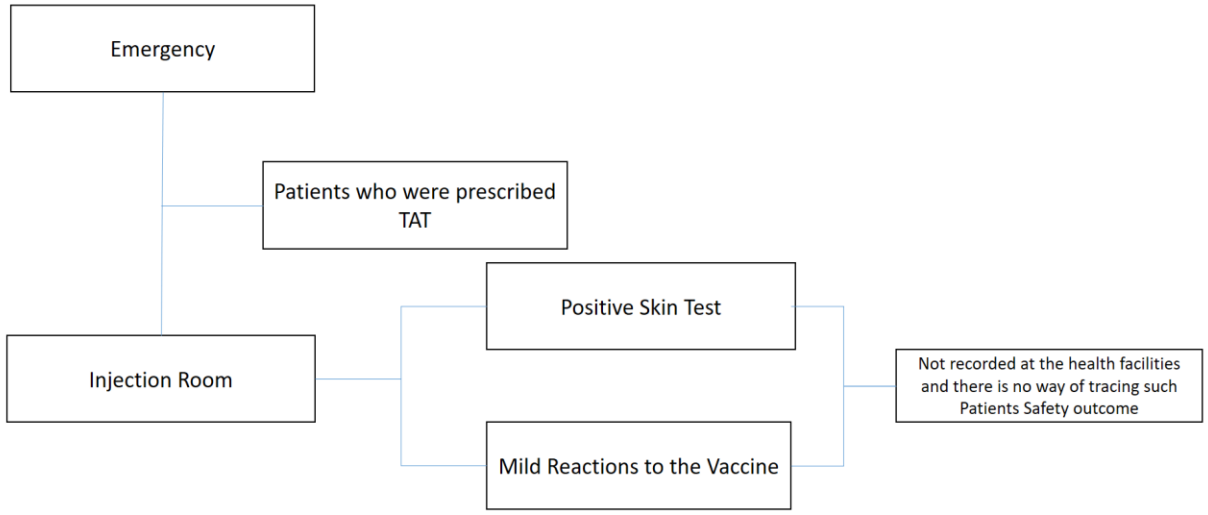

Figure 6. flow chart of health centers for emergency patients

5. Discussion

TAT which is mainly used over TIG in Africa and Ethiopia in view of availability and low cost for post exposure therapy of tetanus [18] causes Adverse reaction in one patient out of 1213 injections.

26.66% medical record forms required TAT injection which was similar to the finding of Sinclair *et al* [34] which showed 600 patients required TAT injection out of total 3000 emergency and trauma visits.

The majority of the participants were males (n=949, 77.08%), which is similar to studies done by J. Lang *et al,* Philipo L. *et al*, Assegid *et al,* Amare A. *et al* and Awoke D. *et al* [19, 27, 35, 36, 37] perhaps for males tend to be more outdoor than females for field and other works [37]

The mean age of the participants was 26.60 +/- 11.59 years old, and this age distribution in this study was similar to the findings by another study's [27], but the median age group which is between 48-57 years old differs from studies done by Keswani NK. *et al*, whose median age was between 16 and 24 years [38] which might be because of the inclusion criteria we used was only for those above 18 years old and unlike the other studies this study included large sample size.

TAT vaccine has to be administered to the patient as soon as possible [39] but in our case 300 (29.03%) patients were given the vaccine after 3 days to even a month. However, there was neither vaccine failure nor disease development history recorded in any of the medical record forms.

Studies done in the developed world showed that people recorded history of active immunization ranges from 22-63.4% [33] which is far higher than our study (8, 0.13%). However, a similar study done in Ethiopia showed that more than 70 % of the study participants had no idea about their active immunization history [19], which is similar with the finding of our study, but on the contrary to our study, 89% of the health facilities had access to TT [24].

A Retrospective study from Tanzania showed that all participants' TT vaccination history was recorded in all patients recording forms which was higher than our finding where only 21 (1.73%) medical record forms had recorded vaccination history, which might be due to poor recording habits that limits a retrospective study in our setting [35, 37].

All study charts used in our study with known history of TT vaccination were those of females which might have a relation with the Expanded Program Immunization which includes TT vaccination and pushes more pregnant women to be vaccinated [40].

Although the exact occupation of the study charts was not indicated, 24% of participants who suffered from trauma were at their work-places which is in agreement with a study done by Berhanu F. et al [41], which indicated 39% work-place related trauma and called for the need for fulfillment of requirements of workplace safety precautions.

Head, hand and leg (lower limb) trauma (n=273, 23.03% and n=270, 22.78 % and n=251, 21.18%, respectively) were the most frequently seen sites of trauma which is similar to the report by J. Lang *et al* and Assegid S. *et al* [27, 36]

A previous single-centre double blind randomized clinical trial conducted in west Africa showed that there was no serious adverse event recorded [27], this finding is partly similar to our study. However, it also found out 16.4% of the participants experienced at least one systematic adverse event which was not seen in our study.

Other studies found that TAT produced adverse events in 5-6% [15, 42], 28% [34] which is sizable difference from the finding by our study (one in one thousand two hundred and thirteen TAT injections.).

Late reactions which will manifest within few days to 21 days' range between 20% to 30% [43], but since our study used retrospective study design, it was not possible to distinguish late reactions like serum sickness which are frequently missed by both professionals and patients, and this might have overestimated the safety of the vaccine under the study.

No death has been reported by our study though studies indicated that there were 0.5 to 2.4 deaths in 10,000 injections [44]. The discrepancy might be associated to the recording culture of the health facilities

According to a study by perey et al, when properly tested, half of the injured people were allergic to the horse serum [15], but no record was found indicating positive skin test. Perhaps patients who were prescribed to take the vaccine were not listed in the injection registration log.

According to the standard for the administration of immunization [45], we have to consider the seven rights, right product, right client, right dose, right time, right route, right reason and right documentation, from these requirements only 11.87% of the medical record forms included in the study indicated the time interval of injection, route of administration and the right dose given.

As can be seen from figure 8 above, the physician in charge cannot know if a mild reaction occurs and the patient reacts positively for the skin test, and it cannot be traced how the patient reacts to the vaccine since it is not recorded on the patients' chart, and this is against the Centers for Disease Control and Prevention (CDC) vaccine Administration guideline [46] which requires the health care providers to record such information in the patient's medical record, and this definitely had an impact on the findings of the study.

One of the requirements, "right documentation" was almost impossible when health facilities kept operating by the provided roadmap, this might have overestimated the safety of the study.

The limitations of the current study include poor recording trends, illegible hand writing on the records and misplacements of documents.

6. Conclusion

With limitations of the current study, the finding suggests that use of equine tetanus antitoxin for prophylactic purpose is safe

7. Recommendations

7.1. Recommendation for researchers

we recommend larger study with wider geographical coverage which will have significance in testing the validity of the current finding, and conducting phase IV clinical trial would be more appropriate to confirm the present findings.

7.2. Recommendation for health workers

Recording every finding and medications given for a patient is very valuable not only for the patient but also for further policy making and research activities.

8. References

1. Berger S. Tetanus: global status. GIDEON Informatics, Inc; California, USA, 2018.
2. World Health Organization. WHO Immunological basis for immunization series; Module 3: tetanus. Geneva, Switzerland; 2018. Available at: https://apps.who.int/iris/bitstream/handle/10665/275340/9789241513616-eng.pdf?ua=1
3. Rhinesmith E, Fu L. Tetanus disease, treatment, management. Pediatr Rev. 2018;39(8):430-32.
4. Dong M, Masuyer G, Stenmark P. Botulinum and tetanus neurotoxins. Annu Rev Biochem. 2018 [Epub ahead of print]
5. Guilfoile P, Babcock H. Deadly diseases and epidemics: Tetanus. Infobase Publishing; New York, NA, 2008.
6. World Health Organization (WHO). Non-neonatal Tetanus: vaccine preventable diseases surveillance standards. Geneva, Switzerland; WHO 2018. Available at: https://www.who.int/immunization/monitoring_surveillance/burden/vpd/WHO_SurveillanceVaccinePreventable_15_NonneonatalTetanus_R2.pdf
7. World Health Organization (WHO). Tetanus vaccines: WHO position paper. Weekly epidemiological record 2017;92:53-76
8. Mallick IH, Winsle MC. A Review of the epidemiology, pathogenesis and management of tetanus. International Journal of Surgery 2004;2:109-12.
9. Kyu HH, Mumford JE, Stanaway JD, Barber RM, Hancock JR, Vos T, et al. Mortality from tetanus between 1990 and 2015: findings from the global burden of disease study 2015. BMC Pub Heal. 2017; 1-17.
10. World Health Organization (WHO). Global and regional immunization profile: Africa region. Jeneva, Switzerland; 2018. Available at https://www.who.int/immunization/monitoring_surveillance/data/gs_afrprofile.pdf?ua=1
11. Altshuler M. Immunizations, an issue of primary care clinics in office practice - E-Book. Philadelphia, USA; Elsevier 2011.
12. Sellon D, Long M. Equine infectious diseases, 2nd ed. Saunders, an imprint of Elsevier Inc. Missouri, USA; Elsevier 2014.

13. Medecins Sans Frontieres (MSF). Essential drugs: practical guidelines intended for physicians, pharmacists, nurses, and medical auxiliaries. Paris; MSF 2016.
14. McCracken GH Jr, Dowell DL, Marshall FN. Double-blind trial of equine antitoxin and human immune globulin in tetanus neonatorum. Lancet. 1971;1(7710):1146-9.
15. Perey BJ. Progress in tetanus prophylaxis: the advent of human antitoxin. Can Med Assoc J. 1966 ;94(9):437-41.
16. Tripathi KD. Essentials of medical pharmacology. New Delhi, India; Jaypee Brothers Medical Publishers Ltd 2013.
17. Rui Yu, Chong Ji, Junjie Xu, Wang D, Fang T, Jing Y, et al. The Immunogenicity of the C Fragment of Tetanus Neurotoxin in Production of Tetanus Antitoxin. Biomed Res Int. 2018;2018:6057348
18. Zhang XJ, Li HL, Deng DY, Ji C, Yao XD, Liu JX. Functional and proteomic comparison of different techniques to produce equine anti-tetanus immunoglobulin F(ab')2 fragments. J Chromatogr B Analyt Technol Biomed Life Sci. 2018;1092:29-39.
19. Derbie A, Amdu A, Alamneh A, Tadege A, Solomon A, Elfu B, et al. Clinical profile of tetanus patients attended at Felege Hiwot Referral Hospital, Northwest Ethiopia: a retrospective cross sectional study. Springerplus. 2016;5(1):892
20. Woldeamanuel YW, Andemeskel AT, Kyei K, Woldeamanuel MW, Woldeamanuel W. Case fatality of adult tetanus in Africa: Systematic review and meta-analysis. J Neurol Sci. 2016;368:292-9.
21. Tadele H. Clinical profile and outcome of pediatrics tetanus: the experience of a tertiary hospital in Ethiopia. Ethiop J Health Sci. 2017;27(5):559-64.
22. Anatea MD, Mekonnen TH, Dachew BA. Determinants and perceptions of the utilization of tetanus toxoid immunization among reproductive-age women in Dukem Town, Eastern Ethiopia: a community-based cross-sectional study. BMC Int Health Hum Rights. 2018;18(1):27.
23. Mamoro MD, Hanfore LK. Tetanus Toxoid Immunization Status and Associated Factors among Mothers in Damboya Woreda, Kembata Tembaro Zone, SNNP, Ethiopia. J Nutr Metab. 2018;2018:2839579.

24. Ethiopian Federal Ministry of Health (FMoH). Ethiopian Emergency Obstetric and Newborn Care (EmONC) Assessment 2016: final report. Addis Ababa, Ethiopia; Ethiopian Public Health Institute 2017.
25. Drug Administration and Control Authority of Ethiopia (DACA). Standard Treatment Guideline for Health centers. Addis Ababa, Ethiopia; DACA 2010. Available at: http://apps.who.int/medicinedocs/documents/s17821en/s17821en.pdf
26. Ethiopian Pharmaceuticals Fund and Supply Agency (PFSA). Pharmaceuticals procurement list. Addis Ababa, Ethiopia; PFSA 2018. Available at: http://www.moh.gov.et/documents/20181/0/PFSA+Pharmaciutcals+Procurement++list+First+Edision/e448b515-53ed-4a24-b71e-b2b6916b2eb4
27. Lang J, Kamga-Fotso L, Peyrieux JC, Blondeau C, Lutsch C, Forrat R. Safety and immunogenicity of a new equine tetanus immunoglobulin associated with tetanus-diphtheria vaccine. Am J Trop Med Hyg. 2000;63(5-6):298-305.
28. Wendt S, Eder I, Wölfel R, Braun P, Lippmann N, Rodloff A. Botulism: Diagnosis and Therapy.Dtsch Med Wochenschr. 2017;142(17):1304-12.
29. Ethiopian Food, Medicine and Healthcare Administration and Control Authority (FMHACA). Good clinical practice guideline. Addis Ababa, Ethiopia; 2018.
30. Alebachew, A., & Waddington, C. (2015). Improving health system efficiency: Ethiopia: human resources for health reforms. *Who*, 1–28.
31. Sahile, A. T. (2020). Medical Record Documentation System in Ethiopia. *American Journal of Biomedical Science & Research*, *10*(4), 375–376. https://doi.org/10.34297/ajbsr.2020.10.001534
32. Wong, R., & Bradley, E. H. (2009). Developing patient registration and medical records management system in Ethiopia. *International Journal for Quality in Health Care*, *21*(4), 253–258. https://doi.org/10.1093/intqhc/mzp026
33. World Health Organization (WHO). Global manual on surveillance of adverse events following immunization. Geneva, Switzerland; WHO 2016. Available at: https://www.who.int/vaccine_safety/publications/Global_Manual_revised_12102015.pdf?ua=1

34. Sinclair, I. S. R., McCormick, J. st G., & Clark, J. G. (1971). Comparative trial of three heterologous anti-tetanus sera. *Journal of Hygiene*, *69*(2), 201–207. https://doi.org/10.1017/S0022172400021434

35. Chalya, P. L., Mabula, J. B., Dass, R. M., Mbelenge, N., Mshana, S. E., & Gilyoma, J. M. (2011). Ten-year experiences with Tetanus at a Tertiary hospital in Northwestern Tanzania: A retrospective review of 102 cases. *World Journal of Emergency Surgery*, *6*(1), 20. https://doi.org/10.1186/1749-7922-6-20

36. Sahilu, A., Kedir, I., & Ayele, Y. (2016). A Four Years Record Review of Tetanus Cases Admitted to Jimma University Specialized Hospital (JUSH), Southwest Ethiopia: A Slip of Tetanus Case Management? *International Journal of Anesthesiology & Pain Medicine*, *02*(01), 1–7. https://doi.org/10.21767/2471-982x.100010

37. Amare, A., & Yami, A. (2011). Case-fatality of adult Tetanus at Jimma University Teaching Hospital, Southwest Ethiopia. *African Health Sciences*, *11*(1), 36–40. https://doi.org/10.4314/ahs.v11i1.64990

38. Abrutyn, E., & Berlin, J. A. (1991). Intrathecal Therapy in Tetanus: A Meta-analysis. *JAMA: The Journal of the American Medical Association*, *266*(16), 2262–2267. https://doi.org/10.1001/jama.1991.03470160094039

39. Sanders, R. K. M., & Peacock, M. L. (n.d.). *regions are inhibited by iontophoretically applied enkephalin , (HORSE) IN THE TREATMENT OF TETANUS Sherrington of intrathecal*. 974–977.

40. Lisboa, T., Ho, Y.-L., Henriques Filho, G. T., Brauner, J. S., Valiatti, J. L. D. S., Verdeal, J. C., & Machado, F. R. (2011). Guidelines for the management of accidental tetanus in adult patients. *Revista Brasileira de Terapia Intensiva*, *23*(4), 394–409. http://www.ncbi.nlm.nih.gov/pubmed/23949453

41. FMOH. (2015). *Ethiopia National Expanded Program on Immunization, Comprehensive Multi - Year Plan 2016 – 2020. Federal Ministry of Health, Addis Ababa, Ethiopia.* 1–115.

42. Berhanu, F., Gebrehiwot, M., & Gizaw, Z. (2019). Workplace injury and associated factors among construction workers in Gondar town, Northwest Ethiopia. BMC Musculoskeletal Disorders, 20(1), 1–9. https://doi.org/10.1186/s12891-019-2917-1

43. Oxytetracycline, A., Toxoid, I., D, R. G. B. M., & Diego, S. (1962). *Tetanus Prophylaxis in the Unimmunized*. *96*(4), 257–261.

44. Meedical, T. H. E. C. (1960). ALLERGIC REACTIONS TO ANTITETANUS SERUM*. 907–912.
45. Alberta Health Services. (2020). *Standard for the Administration of Immunizations*. 1–26. https://www.albertahealthservices.ca/assets/info/hp/cdc/if-hp-cdc-ipsm-standard-administration-immunization-06-100.
46. Center for Immunization and Respiratory Diseases. (2017). *Vaccine Administration Protocols | CDC* (p. 52). https://www.cdc.gov/vaccines/hcp/admin/admin-protocols.html

9. Annexure

a. Annexure 1: Product insert

TETANUS ANTITOXIN
1000/1500 IU IP/BP/IHS

(Each mL Contains Enzyme Refined, Equine Tetanus Antitoxic Immunoglobulin Fragments)

Description

Tetanus antitoxin is prepared by hyperimmunising horses with Tetanus toxoid. Plasma obtained from healthy immunised horses is enzyme refined, purified and concentrated. The Tetanus antitoxin has the specific antitoxic immunoglobulins which neutralize the toxin formed by *Clostridium tetani* the causative organism of Tetanus infection.

Administration and Dosage

Tetanus antitoxin is given prophylactically to persons at the risk of tetanus infection by infected wounds or severe wounds. A dose of 1000/1500 IU should be given intramuscularly or subcutaneously and the dose may be doubled or tripled in case of multiple and severe wounds. Prophylactic dose is also given in surgical operations as post operative care.

Along with this passive immunisation it is advisable to initiate active immunisation with adsorbed Tetanus vaccine.

Serum reaction

In case of patients receiving Tetanus antitoxin, it should be essential to test for hypersensitivity of the individual with a test dose.

Serum sensitivity test is carried out by injecting 0.1 mL Tetanus antitoxin serum in 1:10 dilution either subcutaneously or intradermally and observing for half an hour for any local or general reaction. In case of hypersensitive reaction, serum should be given with great caution in small divided dose subcutaneously at regular intervals of half an hour. Adrenaline Injection (1:1000) must be given for immediate treatment of shock if it develops. Intravenous administration of serum is not recommended in hypersensitive cases.

Storage

The liquid Tetanus antitoxin should be stored at 2°C to 8°C. It should not be allowed to freeze.

Presentation

Tetanus antitoxin is supplied as 1mL liquid in a glass vial/ampoule/pfs.

Disposable

Left over antiserum and used vials/ampoule/pfs should be discarded as Biomedical waste.

Manufactured by:
ViNS BIOPRODUCTS LIMITED
Survey No: 117, Thimmapur (V) - 509325, Kothur (Md), Rangareddy (Dist.), Telangana, India.

Code: 130202084 A.W.No: 15/AAW/PI/02.00 Dt: 25.04.2016

Size: 50x140 mm

b. Annexure 2: Institutional Permission Letter

Study: Safety of Equine Tetanus Antitoxin for Prophylactic Use in Ethiopia: A Retrospective Multicenter Study

Principal Investigator: Prof Eyasu Makonnen

Co-Investigators: Dr. Abebaw Fekadu, Dr Yimtubezinash Woldeamanuel, Dr Girmay Medhin, Dr Tsegahun Manyazewal, Michele Joseph

The research staff of the Addis Ababa University, Center for Innovative Drug Development and Therapeutic Trials for Africa (CDT-Africa) have planned to perform TAT-Safe study to see if the tetanus antitoxin currently in use in Ethiopia is safe.

Tetanus is a bacterial infection that affects the central nervous system. The causative agent, *Clostridium tetani*, is found in the environment irrespective of geographical location. Tetanus may follow puncture wounds, surgery, burns, crush wounds, animal bites, oral infections, or child birth and abortion. Among patients, the case-fatality rate approaches 100% without intensive care. Prophylaxis of tetanus consists of active and passive immunizations. A passive immunization is recommended as post-exposure-prophylaxis within 24 hours after a tetanus prone wound has occurred. The aim of this study is, therefore, to evaluate the safety of equine tetanus which is in use in Ethiopia. You are invited to participate in this study because you administer tetanus antitoxin under your routine clinical care in this health facility.

We have selected your institution to be one of our study site and if your institution agrees to participate in this study, we will kindly ask you to give us permission to review some charts of patients who visited the health care institution for trauma and related cases. Previous medical and vaccination history, reason for administering vaccine, and occurrence of adverse events will be reviewed from the chart.

All clinical, laboratory and other personal information taken from the chart review will be kept confidential. This will be maintained by keeping the hard copies in a locked filing cabinet and the soft copy will be password protected. The study will be frequently supervised and monitored by the investigator and investigators delegate.

The ultimate goal of the study is to ensure Ethiopian citizens are accessing safe and quality-assured medicines

Thank you for your consideration

☐ I give permission to you to conduct the study

☐ I **do not** give permission to you to conduct the study

c. Annexure 3: Data Collecting Tool

Data Collecting Checklist

Chart No: ☐☐☐☐☐☐ Data Collector ID: ☐☐☐☐

Participant's:

Sex: ☐Male ☐Female

Age in years: ☐☐

Residence: ☐Urban ☐Rural

Literacy: ☐Literate ☐ Illiterate

Occupation: ☐No job ☐Student ☐Housewife ☐Trader ☐farmer ☐Gov. employee ☐Daily laborer ☐Other (specify)

Marital Status: ☐Never married ☐Married ☐Divorced ☐Widower

Health and vaccination status:

Weight in No. ☐☐☐

Height in No. ☐☐☐

HIV history ☐positive ☐negative ☐unknown

TT vaccination history ☐Yes ☐No ☐Unknown

Educational Level:

☐None ☐Elementary (0-8) ☐Secondary (9-10) ☐Preparatory (11-12) ☐University (Diploma) ☐University (Degree) ☐University (MSc/PhD)

Nature of Injury

Place: ☐home ☐school ☐street ☐other (specify)

Activity: ☐work ☐education ☐sport ☐traveling ☐others (specify) ☐unknown

Nature: ☐open wound ☐ fracture ☐sprain/strain; ☐cut/bite; ☐bruise; ☐organs system injury

Mechanism:

☐traffic ☐sexual assault ☐fall ☐blunt force ☐stab/cut ☐gunshot ☐fire/heat

Site of injury: specific body part/s injured ……

Time of injury: estimated time from injury onset to clinical visit ……

d. Annexure 4: Reported AEFI and Measures taken Recording Form

Reported AEFI and Measures Taken Recording Form	
Chart No: □□□□□□ Data Collector ID:□□□□ Date Data Collected: _ _ / _ _ / _ _	
Dosage given; **There were AEFI;** Yes □ No □ **Adverse event(s):** □Severe local reaction □Joint Seizures □febrile □afebrile □Abscess □Sepsis □Encephalopathy □Toxic shock syndrome □Thrombocytopenia □Anaphylaxis □Fever ≥38°C Other (specify):	Did the patient visit the health facility within the first 21 days after TAT administration; Yes □ No □ Was it related with TAT Yes □ No □ Unknown □ Mention Findings Measures taken;
Serious: ***Yes / No***; If Yes □Death □Life threatening □Persistent or significant disability □Hospitalization □Congenital anomaly □Other important medical event (specify): **Measures Taken;**	Outcome: □Recovered □Recovered with sequelae □Not Recovered □Unknown □Died: If Died, date of death: _ _ / _ _ / _ _ Autopsy done: □Yes □No □Unknown
Past medical history (including history of similar reaction or other allergies), concomitant medication and other relevant information (e.g. other cases). Use additional sheets if needed:	
Note: Under Measures taken everything recorded should be written	