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Dr. Ranjeet K Singh President International Association of Scientist & Researchers



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Study on the Biological Weapon and their Detection Techniques

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Abstract:

Nowadays, the risk of biological and chemical agents is growing day by day. In these biological agents, certain fungi, bacteria and viruses represent a high risk for public health. Biological agents have the characteristics such as the unpredictable nature and have symptoms similar to common infection due to which the biological agents are used as a weapon by the terrorist to harm the large population. There are different types of methods that has been developed to detect these biological agents. These methods are Electrochemical Impedance Spectroscopy (ESI), Immunoassay techniques, Polymerase Chain Reaction (PCR) and Fluorescence techniques. These techniques have some advantages and disadvantages in the field of biological warfare due to which all biological weapon cannot be detected. In these review paper, we discussed about the different types of technique for the detection of biological weapons and concerns for the future.

Keywords: Biological Agents, Weapon, Detection Techniques.



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Introduction

Nowdays, the awareness is increasing regarding biological attacks vulnerabilities to military areas as well as civilian targets. The objective of homeland defense and national security is to protect the humans and animals against the biological warfare. 'Biological Warfare' means many biological agents that are used as weapons in mass destruction. The characteristics of these biological agents are to infect a large population with minimal economic cost.

From many centuries, the biological agents is used to beat enemies. The use of biological warfare (BW) has a historical record; in world war-I, anthrax biological warfare agent was used by Germans and Japanese against the human beings and animals. Again, it was used on large scale by Japanese against war prisoners and Chinese. The misuse of Biological Warfare Agent (BWA) is a possible event in the increment of threat of terrorist attacks. For a simple and cheap production of biological weapons, the misuse of biotechnology and food industry facilities are the one major problem (Joshi *et al.*, 2013; Trzaskowski and Ciach, 2017).

Biological Agents

A live pathogenic microorganism that are used to cause mass infection or poisoning of people, animals or plants is known as biological agents. The mechanism is as they penetrate in the human body and cause disease. Naturally occurring bacteria, viruses, fungi, rickettsiae and biological toxin are come under the biological agents (BAs).

The several groups of natural sources are divided according to the occurrence i.e. natural occurrence (spores of plants, pathogens coming from bacteria and fungi), agriculture and livestock production, hospital environment and infectious diseases, industrial and other processes. Two natural sources 'Natural and Agriculture' bio aerosols are usually merged with each other as during the handling of hey (straw), inhalation of aerosol or dust, may contain contamination from infected rodents. During the production and storage of grain, problems related to mycotoxins may also be encountered sometimes. Droplet infection is a transmission disease which is not limited to the hospitals, it spreads through the flu and viral infections are found around. There are several reason to use the biological agents as weapons which are:

- **1.** Biological agents (BAs) are multiplied in the human body and give significant effect.
- 2. Biological agents (BAs) are highly toxic and virulent: these biological agents give their

effect after some time, not immediately and transferred from one person to another person easily.

- **3.** Biological agent is a 'Poor man's bomb' because it is inexpensive to construct as it can be grown in facilities such as pharmaceutical, food and medical production sites. There is time gap between infection and appearance of symptoms in the absence of adequate detection systems that gives the perpetrators a chance to escape.
- 4. The dose for infection in biological agents is very low as compare to other types of agents (like chemical agents). Samples of biological agent should be in milligram (mg) to gain the desired result (Joshi *et al.*, 2013; Svabenska, 2012).





Classification of Biological Agents

Bio agents have been divided into five categories:

Bacterial Agents: - It is small, single-celled organism that grows on solid or liquid culture media. Different types of diseases are produced by bacterial agents are Anthrax, Tularemia, Cholera, Diphtheria, plague and typhoid fever.

Viral Agents – It is simplest type of microorganism. It contains genetic material (either DNA or RNA) with coated protein. Different types of disease caused by

viral agents are smallpox, yellow fever, dengue fever and Ebole.

Rickettsiae – It is obligate intracellular bacteria which have the size between bacteria and viruses with the common characteristics to both bacteria and viruses. Different types of diseases caused by rickettsiae are Qfever, Endemic typhus and Rocky Mountain spotted fever.

Fungi – It may be unicellular or multicellular microorganism. It does not contain chlorophyll. It is generally used to destroy crops rather than human. Coccidioidomycosis disease caused in human by fungi.

Biological Toxin – It is a poison, not any organism that give adverse effect on humans. Biological origin toxin are Botulinum, Toxin, Saxitoxin and Ricin (Joshi *et al.*, 2013).

The effective protection against Biological warfare agents is difficult because of the detection of their presence is complicated and expensive. The biological agent infection is similar to the common infection due to which medical treatments are unsuccessful. After several studies, many devices are used for the detection and identification of biological agents. The point detection devices are the most available systems that can be used in field as well as at laboratory level but most of the device are commonly found in laboratories that are used for identification purpose. The detection and identification equipments of biological agents can also be stored on the basis of different fundamental principles (Svabenska, 2012).

Review of Literature

Svabenska (2012) gave a conclusion on the basis of his review, many factory made system for the detection of bio aerosols are generally used at hazardous places such as airports, subways, stadium, state buildings and semi enclosed facilities. He discussed in his paper about integrated detection system, spectroscopy and optical. There is need of trained operator for handling the devices except one hand held systems. Sample preparation is also an important steps in the detection and identification.

Joshi *et al.* (2013) discussed in their paper about biological agent 'Bacillus Anthracis' and their detection by Ultra violet Laser Induced Fluorescence (UV-LIF) spectroscopy method in which Intensified Charge Coupled Device (ICCD) spectrometer and Multi Anode Photo Multiplier Tube (MAPMT) were used for the detectors. They concluded in the end of paper that MAPMT based system gives better result **as** compared to ICCD in the term of sensitivity and UV- LIF-LIDAR system is best for the standoff detection of biological aerosols.

Ducote *et al.* (2016) proposed a method Electrochemical Impedance Spectroscopy (EIS) for the detection of pathogen 'ricin' (biological agent) and concluded that ESI is a highly sensitive, real time and non-destructive method for the detection of cytotoxin. Future perspective also discussed by Ducote et al as this technique can have the effective result on other toxins different from ricin in both case structure and mechanism of action.

Tomar *et al.* (2016) studied on the botulinum neurotoxin (BoNT/A) using surface plasmon Resonance (SPR) with carboxymethyldextran modified sensor chip. Electrochemical Impedance Spectroscopy (EIS) data were used for the confirmation of an effective interaction between the antigen and antibody. Different values were noticed such as limit of detection (LOD), kinetic parameter and Bmax values of immobilized antibody and immobilized synaptic vesicles. The main aim of this study was to give the inputs in the development of SPR based sensors by the help of antigen and antibody interaction for BoNT/A, and other BWAs.

Rowland *et al.* (2016) give an outlook of paper and concluded that nanomaterial based sensors techniques are more effective than the traditional techniques because of the high sensitive power and accuracy.

Trzaskowski and Ciach (2017) used Surface Plasmon Resonance (SPR) technique for the detection of biological agents. They concluded that the sensitivity of this method depends upon the type of biological agent. The detection limit is also different for each sensing chip that starts from 50 cfu/ml for V. cholerae bacteria. This technique, Surface Plasmon Resonance can be useful for the detection of biological agents.

Saito et al. (2018) in their paper 'Field-deployable rapid multiple bio sensing system for detection of chemical and biological warfare agents' discussed about the collection and detection method for the biological agents. They developed an autonomous air sampling and detection system. The collection of biological and chemical agents was possible using designed collection system. Detection system 'ESI' is able to detect the concentration lower than the mean lethal dose, toxic proteins by Localized Surface Plasmon Resonance and Chip PCR is used to detect the Pathogens. Within 5-15 minutes, collection and detection of the biological and chemical agents is completed. In this research, they developed a compact and battery operated device that have all unit into on system.



Duracova *et al.* (2018) discussed in their review about the protein toxin and its detection methods. There are different types of methods have been developed such as immunochemical assays, MS based method, chromatographic method etc. But there is need to improve the state of detection in different ways such as upgrading the pre-analytical phase, suitable reagent and so on. They concluded that protein toxin cannot be detected by only one technique, a combination of techniques should be used to obtain the most information for complex samples.

Conclusion

The biological agents are the microorganism that are used as a weapon in war or by terrorists for harming

the common people in large amount. Different methods have been developed which played a vital role in the detection of biological and chemical weapons which ultimately help the first responder and medical examiner in detecting the agent and then, cure the person. In spite of using these techniques and running programs (Biological Weapon Convention) against the biological warfare, the attacks of biological weapon are not under control. So, this review study concludes that there is need to develop more advanced techniques or implementation in the existing techniques to detect these weapons.

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Study on Diagnosis of Turner Syndrome

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Abstract:

Turner's syndrome (TS) is referred to as the monosomy X, in which total or partial loss of one sex chromosome (45X) with the ratio of 1:2500 in live born infants with phenotypic females. Commonly known as Ulrich Turner Syndrome and it contain main clinical features that includes short stature, cardiac anomalies, lymphedema, primary ovarian, gonadal dysgenesis, swollen hands and feet, webbed neck and neurocognitive difficulties. Patients face various difficulties during increasing over the lifespan in given complexity of the condition. The main problem is because of the delay in diagnosis of Turner's syndrome, as only 15-30% patients are diagnosed during the first year of life. The diagnosis and care of turner syndrome was published on individuals in 1994. By the knowledge of complex etiology and detailing more about its clinical variability and difficulties conclusively allow us to develop the therapeutic and management approach of such patients. In this review paper, study about the diagnostics changes of the turner syndrome in individual.

Keywords: Turner Syndrome, Diagnosis, Etiology



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Introduction

Turner's syndrome, is partial or complete loss of second X-chromosome in new birth females (Ramirez and Villarreal, 2016). This syndrome is not inherited by parents, normal people contain the 46 chromosome but inherited people have 45X chromosome (Kadakol et al., 2017). It is designed by Henry Turner in 1938 which included the short stature, sexual infantilism, cubitus valgus and pterygium coli (Shankar and Backelijauw, 2018). Ford et al. recognized the syndrome's chromosomal base in 1959 and find out that presented 45X chromosomes in patients with single X chromosome. It is occur in one every 2500-3000 live birth and it is only full monosomy which is compatible with life (Ramirez and Villarreal, 2016). Common issues was recognized in Turner syndrome that is Short stature, pubertal delay/ovarian insufficiency, cardiac and renal abnormalities, sensorineural hearing loss, ophthalmologic problems, abnormalities. metabolic thyroid syndrome, inflammatory bowel disease and neurocognitive issues (Shankar and Backelijauw, 2018). Turner syndrome with mosaicism is defined as the chromosomal abnormality may be present in some cells. This syndrome was found in 1-2% pregnant ladies and 99% have a spontaneous abortion. About 60% cases of turner syndrome that has 45X chromosomes, 5-10% cases show X chromosome anomalies which is deletion of long or short arms, isochromosomes or ring chromosomes and 6-9% cases shows a normal or structurally Y chromosomes (Iqbal, 2014).

This clinical review is focused on the latest update of diagnostic for the most common clinical concerns that is related to turner syndrome (Pinsker, 2012). It is improved the recognition of these comorbidities and their management by using recent diagnostic (Shankar and Backelijauw, 2018).

Diagnosis

Turner syndrome's diagnosis can occur at a wide range of age (Shankar and Backeljauw, 2017). In the function of clinical and hormonal findings, Diagnosis is suspected. By using a conventional Karyotype or other cytogenetic analysis, confirm the diagnosis of a subject aneuploidy. In case of 3-6% women, with turner syndrome that have a 45X/46, XY mosaic and with the risk of presenting gonadoblastoma during their lives lies between 7% to 30% (Ramirez and Villarreal, 2016).

Diagnosis was occur into two forms:

- Prenatal Diagnosis
- Postnatal Diagnosis

Prenatal Diagnosis

Prenatally, diagnosis system is increase in turner syndrome but exists the significant ascertainment partiality in that underlying reason for prenatal chromosome analysis often impacts the validity of the findings. Ultrasound findings in prenatal turner syndrome that include the nuchal translucency (result is fairly specific), cystic hygroma (ultrasound findings alone can predict turner syndrome in 30-70% cases), coarctation of the aorta and/or other left-sided heart brachycephaly, defects. malformations. renal anomalies, polyhydramnios, oligohydramnios, and growth retardation. In both (nuchal translucency and cystic hygroma) case, ultrasound finding should be seen in autosomal trisomy syndromes and finding appears in which specificity for turner syndrome depends on gestational age. Due to specific ultrasound finding, 45X fetuses are discovered, "classic" phenotypic findings are likely.

With the increasing of ultrasound finding, prenatal counseling is important because the loss of rate of spontaneous fetal for 45X fetuses. The quantity of TS occurring is as many as 3% of all the fetuses and hence later can cause around 10% of spontaneous loss of fetus including 99% of 45X embryos terminating spontaneously during the first and second trimesters.

60% of turner syndrome fetuses are electively terminated in some countries. An explanation that even with an ultrasound finding, delivery of a viable newborn is possible and many of those children go on to have an excellent quality of life included in prenatal counselling.

False positive results can occur, when a prenatal karyotype is performed for other reasons such as advanced maternal age or abnormal maternal screening tests. The fetus with 45X karyotype or loss of one chromosome in newborn phenotypic female finding then diagnosis incidentally. Fetus have not only fewer phenotypic finding but the result of karyotype can be non-specific in case of discovered mosaic karyotype.

Diagnostic information is received by the highresolution ultrasound and fetal echocardiography. Fetuses with turner syndrome are detected by using the maternal biomarker or maternal plasma DNA sequencing but is still preliminary stages (Pinsker, 2012).

Postnatal Diagnosis

During infancy, Lymphedema is the most common disease that is seen in 97% cases while short stature is most commonly leads to evaluation during childhood and adolescence that is seen in 82% cases. Ychromosomal material may be present in 5% individual which have a marker chromosome that is revealed by the analysis of karyotype. Current guidelines advocate screening for Y material if signs of virilization develop or a marker chromosome has already been identified, because the risk of developing gonadoblastoma with Y material present ranges from 5–30% in recent studies.

Earlier age of diagnosis, delay in the diagnosis of turner syndrome is shown by retrospective analyses, averaging 5 years after patients had fallen below the 5th percentile in height to time of diagnosis. 20% patient are diagnosed after the age of 12 years and important questions are arise that how to diagnosis of turner syndrome earlier. Earlier diagnosis, if diagnosis could be done non-invasively or as part of newborn screening that should be allow for detection of cardiovascular and renal anomalies which is unidentified until the time is diagnosed and facilitate for treatment of growth failure.

The value of high through put pyrosequencing of buccal swabs for turner syndrome is the recent advances testing. By using pyrosequencing, quantitate relative allele strength, readily detect loss of an entire X-chromosome or mosaicism with up to 97% sensitivity in this testing. This technology is very useful for non-invasive screening for turner syndrome (**Pinsker, 2012**).

Review of Literature

Hjerrild, Mortensen and Gravholt (2008), in this paper, patients are needed to comprehensive care from a multidisciplinary team whose are suffer from turner syndrome and team done the best practice from outpatient clinic with special importance on turner syndrome. Related to turner syndrome knowledge is limited, this syndrome is infrequently seen by clinicians and patients that have a range of questions related to syndrome.

Pinker (2012), up to 30% of cases of turner syndrome are diagnosed prenatally which is showed a normal karyotype at delivery that is done by cytogenetic registry. This study is complicated by the fact that mosaicism is common finding in chorionic villus sampling or amniocentesis. In this review paper, improvement in the diagnosis of growth failure, cardiac disease and ovarian failure for the care of women with turner syndrome.

Iqbal (2014), stated that multidisciplinary care involving newborn screening, regular cardiovascular examination, GH and estrogen supplements with appropriate pubertal development had changed the scenario, with the hope of giving them a normal quality of life.

Bondy (2014), using newer genetic screening tests for the diagnosis of postnatal turner syndrome that requires further validation, with the 20-30 cell karyotype remaining the gold standard.

Ramirez and Villarreal (2016), turner syndrome is a chromosomal syndrome, in which partial or complete loss of second sex chromosome in newborn female which may cause a sub-diagnosis of its cases. It is most important that know the clinical and genetic aspects which will lead to a timely detection of cases and proper management of co-morbidities of genetic condition.

Yang (2017), stated that marked advances in diagnosis and first treatment of diagnosis in 1939 by Henry Turner. For diagnosis, feasibility of molecular technique needs to be further established. In young adults with turner syndrome, improve quality of life by development of normal height attainment and age appropriate pubertal.

Culen *et al.* (2017), in this review paper, growing the upcoming or expected difficulties with turner syndrome. The aim of this review, provided the treatment of girls with turner syndrome through the help of psychologist or other healthcare providers with challenges. It is conducted that a multidisciplinary standard of care based on a well-defined screening would greatly improve the long term outcomes of patients with turner syndrome.

Kadakol (2017), clinical and structural changes in chromosomes with turner syndrome like Y chromosome mosaicism. Type of genetic disorders is the essential study of chromosome, which will turn in help for diagnosis.

Shankar and Backeljauw (2018), turner syndrome's guidelines are based on expert consensus and evidence for optimal hormone replacement throughout the age spectrum in turner syndrome is still evolving. Patient's health and quality of life related to turner syndrome are improved by adequate support and medical care.

Conclusion

Turner syndrome is a mutation due to which many diseases are occur such as short stature, webbed neck, pubertal delay/ ovarian insufficiency, cardiac and renal abnormalities, sensorineural hearing loss, ophthalmologic problems, thyroid abnormalities etc. This syndrome is improved by giving the clinical and medical health care of patients by best practice of medical and psychologist. Postnatal turner syndrome (a type of turner syndrome) is diagnose by using the

screening test, it is the recent technology. It is conducted with a multidisciplinary standard of care based on a well-defined screening, would greatly improve the long term outcomes of patients with turner syndrome.



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Study on Umbilical Cord Blood Banking for Stem Cells and its Associated Therapeutic Uses

Durgesh J. Pandey¹

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Abstract:

In current scenario, number of fatal diseases are increasing drastically which can not be cure by normal treatments. These fatal diseases now can be treated by umbilical cord blood, the richest source of haematopoietic stem cells. Stem cells have the potency of converting itself into any type of organ specific cells and then forming organ specific tissues therefore helpful in healing the damaging tissues or curing the fatal diseases. The present study is discussing about the umbilical cord blood banking for stem cells and its associated therapeutic uses. Collection and banking of umbilical cord blood (UCB)-derived cells have become a popular option worldwide. Cord blood banking is the preservation of the umbilical cord blood which is collected just after the baby birth. Due to the involvement of the professional organizations and their published standards, UCB banking has very much improved with the passage of time.

Keywords: Stem Cells, Umbilical Cord Blood, Cord Blood Banking, Pluripotent, Therapeutic Uses.



1. Lucknow University, Lucknow

Introduction

In the world of new findings and around more than 130 million global annual births, Umbilical Cord Blood (UCB) is the most plentiful source of the stem cells having the potential of regeneration for clinical significance. It provides effective cure by the spectrum of diseases and mostly used against the blood disorders (Roura *et al.*, 2015). Although the technologies have been advanced in the field of stem cells, still there are many people unaware about the stem cells, and its technologies, developed for the health care worldwide.

Stem Cells

In 1900s, Maximow proposed that the lymphocytes migrated through tissues to form blood circulation components and acted as common stem cells. In 1960s, the research on stem cells cleared its beginning as known today. Stem cells can be defined as the cell having the capacity to divide and make its copy or may transform into any type of cells. Stem cells can be classified as totipotent, pluripotent, multipotent or unipotent depending on its potency. Stem cells can be categorized according to its source of origin that from which tissue it is obtained named as Embryonic Stem Cells, Adult Stem Cells, Induced Pluripotent Stem Cells, Neonatal Stem Cells and Umbilical cord blood stem cells.

The umbilical cord as a source of stem cells

Since 20 years, umbilical cord blood has been used clinically as a source of haematopoietic stem cells transplantation. The embryo attached via connecting stalk to form placenta from the third week of developmental process which transforms into the umbilical cord afterwards. The stem cells can be provided by the umbilical cord through blood running in the umbilical vessels, walls surrounding the vessels and from the Wharton's jelly, which are collected at the time of birth using sterile collection kit having anticoagulant (usually citrate or heparin) in it (Forraz and McGuckin, 60-62).

Umbilical Cord Blood Banking

Umbilical cord blood are thought to be very much helpful in curing thr number of genetic diseases, cancer, blood malignancies, inherited metabolic disorders, bone marrow failures and immune deficiencies, as it is the rich source of stem cells. UCB contains many life saving cells therefore it is very useful to preserve them by UCB banking. Main significance of the UCB banking is to treat the genetic diseases which cannot be treated by the normal treatment. UCB donors need to sign the consent form before collecting the blood. Collection can be done by simple matter of venipuncture and drainage to the sterile container (Roura et al., 2015). Cord blood is collected in collection bag using gravity, when the placenta is still inside the uterus by puncturing the umbilical vein (Sivakumaran et al., 163). After the collection, blood is sample is place in sterile bag of approximately 250 ml in size and place in extraction kit in which temperature, pH, CO₂ and O₂ levels should be depend on the the time and external conditions (Roura et al., 2015). The storage and transport temperature should be maintained at $22^\circ \pm 4^\circ C$ and blood must be labeled, volume recorded and weighed excluding the weight of bag (Butler and Menitove, 2011). For the cryopreservation of the cord blood, UCB is placed in an automated microprocessorcontrolled rate freezer. The cold freezing cryopreservative solution containing 60% DMSO should be added after the WBCis chilled. Cryopreservation of methodology involves 1°C/min cooling down to -60°C, and then a drop to -120°C, 5°C/min. At last of this procedure it must be stored in liquid nitrogen freezer (Sivakumaran et al., 163).

Therapeutic Uses of Umbilical Cord Blood

UCB of human provide unresolved health treatment because of having rich source of hematopoietic stem cells, totipotent cells and pluripotent cells. Cord blood is termed as regenerative medicine as it has the capability to develop into organ specific cells to form organ specific tissues. Some fatal diseases such as cancer Alzheimer's, Arthritis, Asthma, Diabetes, Heart disease, Strokes and blood disorders can be completely cured by UCB. Following are some examples of diseases treated with UCB transplant.



Cancers	Blood Disorders	Congenital Metabolic	Immunodeficiencies
		Disorders	
Acute Lymphocytic	Sickle-Cell Anemia	Adrenoleukodystrophy	Adenosine Deaminase
Leukemia			Deficiency
Acute Myelogenous	Fanconi's Anemia	Gunther's Disease	Wiskott-Aldrich's
Leukemia			Syndrome
Chronic Myelogenous	Thalassemia	Gaucher's Disease	Duncan's Disease
Leukemia			
Myelodysplastic	Evan's Syndrome	Hurler's Syndrome	Ataxia-Telangiectasia
Syndrome			
Neuroblastoma	Congenital Cytopeni1a	Hunter's Syndrome	Digeorge's Syndrome
Hodgkin's Disease	Aplastic Anemia	Krabbe's Disease	Myelokathexis
Non–Hodgkin's	Diamond-	Sanfilippo's Syndrome	Hypogammaglobuline
Lymphoma	Blackfananemia		Mia
Burkitt's Lymphoma	Amegakaryocytic	Tay-Sachs' Disease	Severe Combined
	Thrombocytopenia		Immunodeficiency

Table 1: Examples of Diseases Treated with UCB Transplant

Source: (Sivakumaran et al., 166, 167).

Review of Literature

Forraz and McGuckin, 2010 proven that the placental and umbilical derived tissues must be considered for use. There is no ethical controversies in cthe collection of these umbilical cord only is the matter about is potential for defined clinical trials. The also stated that government need to be ready for the cell therapy to allow rapid transition of cells to hospital and clinics.

Butler and Menitove, 2011 continued to calculate the efficacy of cord blood cells in the treatmet of human diseases including heart diseases, strokes, brain or spinal cord injuries and cancer. The review gives the summary of status of umbilical cord blood banking, history and current uses in curing the human disease.

Ding *et al.*, **2015** concluded that Human umbilical cord mesenchymal stem cells has many effective advantages such as it is a noninvasive collection procedure and have lower risk of infection. It has also a nontumorigenesis, multipotency and low immunogenicity. Although it is best for the clinical use, still not known.

Lopes *et al.*, 2016 identified factors related to the quality of UCPB specimens defined best method of their collection within an UCPBB. According to them,

there is need to develop and improve the technical skills of the obstetrical team. The practices listed in this study is proved to be helpful in obstetrical professionals.

Nasadyuk, 2016 studied at the objective umbilical cord stem cells: biological characteristics, approaches to banking and clinical application and stated about the importance of umbilical cord in clinical applications and biobanking.

Kowsari *et al.*, **2017** concluded that in future there will be vast need and demand of the umbilical cord blood stem cells. It is demonstrated by the recent medical progress that these cells could be used to treat the disease because the haematopoietic stem cells are available in bone marrow. These cells are now being used for the treatment of approximately 80 diseases including cancer, immune deficiencies, cardiovascular diseases, neurological disorders and blood diseases.

Babu, 2017 discussed in his review study that stem cells plays an important role for regenerative medicine and contribute in natural healing of damaged tissues and give a huge amount of therapeutic potential. Study on treating more diseases with cord blood is still going on by many scientists like many researchers are doing trials for cerebral palsy and hypoxic ischemic encephalopathy by using patient's own cord blood.



Conclusion

The present study is on review study of umbilical cord blood banking for stem cells and its associated therapeutic uses. In this review, it is discussed that umbilical cord blood banking is very much significant for the need of stem cells in future for curing fatal diseases. There are many therapeutic uses of umbilical cord blood in the treatment of genetic fatal diseases which are also enlightened in present review study.



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Bacteriological Study of Diabetes Foot Ulcer

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Abstract:

Diabetes is a long life disease in which the sugar level is increased in blood. Diabetes contains the one of the most complication disease that is Diabetic foot ulcer. Diabetes foot ulcer is one of the major medical, social and economic complications of Diabetes mellitus and this infection has polymicrobial nature. Diabetes foot ulcer infections have the optimal treatment in which the type of foot ulcer infection is recognized and pathogen-appropriate antibiotic therapy is suggested. In case of non-recognizable and uncontrolled of foot ulcer diabetes, it can be leads to many devastating consequences like limb amputation, sepsis, and even mortality and hospitalized. In this review paper, we studied about the bacteriological profile of Diabetes Foot Ulcers.

Keywords: Diabetes Mellitus, Foot Ulcer Diabetes, Polymicobial, Pathogen





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Introduction

A diabetic foot is one of the most complicated of diabetes that leads to cause hospitalization among diabetic patients (Shanmugam, Jeya and Linda, 2013). According to World Health Organization (WHO), this disease widespread with an increasing incidence and peoples are afflicted approximately 150,000,000 across the world. Diabetic Foot is referred to as infection and ulcers that is accompanied by neuropathy and arteriovnous abnormalities in the foot of patients with diabetes (Sheikh et al., 2014). Neuropathy, peripheral vascular disease, foot ulceration and infection with or without osteomyelitis are pathological complication that is diseases of Diabetic Foot Disease and this disease leads to the development of gangrene and necessitates limb amputation. Approximately 57 million Indian peoples will affected by Diabetes in the year of 2025. Individuals contain diabetes have at least 10 fold greater risk that leads to being hospitalization for soft tissue and bone infection of foot other than without diabetes (Shanmugam, Jeya and Linda, 2013).

In patients, damaging of the micro-vascular circulation with a diabetic foot limits the access of phagocytes due to which development of an infection increases. In lower extremities, the blood supply is compromised by local injuries and improper foot wear but foot infection with diabetes are initially treated empirical in patients, a therapy which is directed at the known causative organism may improve the outcome (Sheikh *et al.*, 2014).

Many studies have been reported before 25 years ago on bacteriology of Diabetic Foot Infections (DFIs) but its result have been diverse and contradictory. The differences in the positive organism causes discrepancies, which had happen overall period of time, variation in a geographical region on the infection type or severity as were stated in various studies. Bacterial infections are mixed by diabetic foot infection and requires the proper management of these infection by an appropriate antibiotic selection, based on the culture and the antimicrobial susceptibility testing (Shanmugam, Jeya and Linda, 2013).

Based on severity and depth of ulcers, different microorganisms are isolated from diabetic foot infections. In superficial ulcer, gram-positive cocci are the most common germs but anaerobic bacteria are mostly found in deeper lesions. The existence of different type of microorganism along with the growing resistances to antibiotic therapy has literally compromises the therapy of empiric in diabetic food infection (Sheikh *et al.*, 2014).

Important contributor to the chronicity of wounds are the diversity of bacterial populations in chronic wounds such as diabetic foot ulcers that are beginning with the medical and research communities. Major populations of bacteria are examined that were associated with the bio burden of infected diabetic foot ulcers. Survey was performed on the wounds in which identification of the genera or the noted pathogens that were present in the diabetic ulcers. This survey also notes down the changes in the bacteriological profiles of the infected foot ulcers and comparison was done from previous studies (Shanmugam, Jeya and Linda, 2013).

Polymicrobial infection involving gram negative and obligate anaerobic organisms are likely to occur in other patients. For the treatment of diabetic foot ulcers, using the antibiotic therapy in which need to be guided appropriately in the light of causative organism and its sensitive pattern to various drugs and calls upon a well-planned bacteriological study of diabetic foot ulcers (Patil and Mane, 2017).

Currently, there is a paucity of data on the ESBL that is carbapenemase producing organisms from diabetic foot infections, especially in this part of world (Shanmugam, Jeya and Linda, 2013).

Treatment of Diabetic Foot Ulcer

Controlling the hyperglycemic burden whose patients presenting with the Diabetic Foot Ulcer (DFU) by calling up to "the Triad". This triad is a wide spectrum antimicrobial chemotherapy including a 3rd generation Cephalosporin Ceftriaxone, a 2 generation fluoroquinolone and Ciprofloxacin and Lincosamide class, Clindamycin. This triad was given together with a periods of two weeks.

In case the required result are not achieved with the triad then, decision of doing a culture and sensitivity test for all patients before starting the empirical antibiotic Triad is done.

Our Inclusion Criteria Included:

- Without osteomyelitis, positive diagnosis of infected diabetic ulcer.
- During 3-months periods, ability to attend the clinic visits.
- Lab tests confirming active infection (Complete Blood Count (CBC) with high Thin Layer Chromatography (TLC), Erythrocytes Sedimentation Rate (ESR) and C-reactive Protein (CRP))
- Acceptance for a written consent

The Exclusion Criteria were:

- According to Infectious Diseases Society of American Classification, patients with severe infection causing remarkably disability.
- Presence of Osteomyelitis.
- Patients with moderate to severe renal impairment.
- Patients with moderate to severe Peripheral Arterial Disease (PAD) that was clinically diagnosed by absence of both distal pulse and confirmed by Duplex study.

Antibiotic Susceptibility Testing: By using disc diffusion method, all bacteria isolates were tested for antibiotic susceptibility against selected members of the following groups: Amikacin, Gentamycin, Clindamycin, Amoxicillin/ Clavulanate, Azithromycin, Ceftazidime, Cefotaxime, Cephalexin, levofloxacin, Ciprofloxacin Ofloxacin, Piperacillin/Tazobactam, Dicloxacillin, Ipipenem, Ampicillin/sulbactam, Chloramphenicol and Penicillin. According to Kirby-Bauer technique, measuring the diameters of inhibition zones in millimeters for estimated the sensitivity (Sheikh et al., 2014).

Methicillin-resistant Staphylococcus Aureus (MRSA) Detection: By using a cefoxitin (30 µg) disc, phenotypic test for the detection of MRSA was done. In case of equal to or more than 22mm of cefoxitin then organism was reported to as Methicillin Sensitive Staphylococcus aureus while cefoxitin is less or equal to 21mm were reported as Methicillin Resistant Staphylococcus aureus (MRSA) (Shanmugam, Jeya and Linda, 2013).

Review of Literature

Citron (2007) dictated that failure to treat appropriately patients with these potentially limbthreatening infections can result in a poor outcome. This study showed moderate to severe diabetic foot infection in patients that are not received antibiotic therapy, specifically these study are polymicrobial with mixed gram negative and gram positive species and in average form, 2.7 aerobic bacteria and 2.3 anaerobic bacteria per culture-positive specimens.

Alsaimary (2010) concluded that greater found of aerobic and anaerobic bacterial infections/pathogens from diabetic patients. These paper are finding that lycos in which increase the risk by abnormally high levels of blood sugar in diabetic patients which damage the blood vessels, causing to thicken and leak etc. Poor circulation result is leads to ulcers that is located in the feet, these ulcers are slow to heal and frequently become deep and infected. This study done the comparison of bacterial wound infection in diabetic patients with non-diabetic patients. It's indicated the high blood sugar can increase infection rate and impair wound healing and wound inflammation and infections can elevate blood sugar.

Shanmugam, Jeya and Linda (2013) in this review paper, diabetic foot infections are caused by both gram positive cocci and gram negative bacilli and show the greater importance of gram negative bacilli. The pattern of antibiotic susceptibility are isolated from diabetic foot infections that is crucial planning for treatment of this disease.

Sheikh (2014) stated that identify the bacterial pathogens that is associated with diabetic foot ulcer and find out the antibiotic susceptibility pattern in a limited number of patients. Within the 3 months of period, improve the rate of ulcer (through various criteria). Form diabetic foot infections, knowledge of antibiotic susceptibility pattern of the isolates that is imperative for planning of the appropriate treatment.

Simonsen et al. (2015) stated that hospitalization as well as infections treated in outpatient setting is caused by the infectious disease. In this review paper, observed that bacterial infections were more common in patients with type I diabetes. Reported use of antibiotics and frequency of bacterial infection were basically associated in diabetic patients having an increased risk of incident microalbuminuria. It show the result with type I diabetes that increase the risk of less severe infections, can be treated outside the hospital. It is observed that diabetic nephropathy at all stages as a risk factors for bacterial infections. Microalbuminuria did not increase the rate of hospitalization due to bacterial infections but in case of macroalbuminuria, double the rate that is compared with patients with normal AER.

Jneid *et al.* (2017) stated that difficulties of differentiating infection from colonization, importance of accurate sampling and transport to laboratory and limits of both culture and molecular based methods to give a good representative of the pathogenic bacterial burden. In this review paper, not only include bacteria but also includes viruses, protozoans and fungi attached to biotic surfaces that display specific inter-microbial and host interactions.

Patil and Mane (2017) within the population of people, high occurrence of foot ulcers with diabetes. Foot ulcerations may lead to infection, lower extremity amputation and cause of disability to patients which result in significant morbidity, extensive periods of hospitalization and mortality. A provided of high standard of care and appreciation of causative organism in diabetic foot.



Conclusion

Foot ulcers occur at high level within the population of people with diabetes. It leads to infection, lower extremity amputation and are the major cause of disability to patients that resulting in morbidity, extensive periods of hospitalization and mortality. These diabetic foot infections are caused by both gram positive cocci and gram negative bacilli. This review studies conclude that there is need to provide the high standard of care and appreciation of causative organisms in diabetic foot. The treatment of diabetic foot ulcer infection is done by the study of antibiotic susceptibility pattern of isolates from the diabetic foot infection.



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Species Identification from DNA Barcoding Technology

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Abstract:

DNA barcoding is a taxonomic method that identify the species in fast and accurate way. It is the method that uses the short DNA fragment, generated from the standard region of genome for the individualization. It works as a new biological tool in organismal biology to increase the understanding about natural world. DNA barcode technique is used in all prokaryotic and eukaryotic plants and animals. Different types of barcode has been discovered that are used for the species identification. In animal, cytochrome oxidase I (COI) is used while in plant different types of barcodes such as rbcL, matK, trnHpsbA, ITS2 and so on. These barcodes have the limits as they are used for the particular species. In this technology, BLAST (Basic Local Alignment Search Tool), and BOLD (Barcode of Life Database) are used to maintain the data of museum and herbarium species and used as a standard for the comparison purpose. In this review study, the main objective is to find out the ability of DNA barcoding for the species identification.

Keywords: Barcoding, Species, BLAST, BOLD



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Introduction

For the species level identification, DNA barcoding concept is very popular. It is a taxonomic method used to identifying the organism and its species. In DNA Barcoding, a short genetic marker is used to identify DNA of an organism because of the specific variation between short DNA sequences from a uniform locality of genome. Unknown sample in the pre-existing classification is identified by the help of DNA barcoding and also determines whether unknown species in sample is separated or combined. Different barcode region are used: segment of mitochondrial gene cytochrome oxidase I (COI) contain 600 base pairs, a barcode region is used in case of animal. It is also used in plant to identifying the leaves because of the absence of flowers and fruits. A portion of plastid gene rbcL codes for the larger unit of Ribulose-1, 5bisphosphate carboxylase (RuBisCO) and Maturase K gene (matK), Standard DNA barcode with the additional regions can be used for the plant, proposed by Plant Working Group, CBOL in 2009. But rbcL gene reported as low discriminating power. Now, a huge number of reference DNA barcode sequences have been generated from taxonomically authenticated species. Hence, the identification of species from any unstructured plant part can be made from the similarity search with reference database (Sarvananda, 2018; Mahadani and Ghosh, 2013).

DNA Barcoding Method

Two basic steps are required for the process of DNA barcoding.

- 1. Make a barcode library of known species- A taxonomic expertise is required in selecting one or several individuals per species to serve as reference samples. First, DNA barcodes for texa already housed in museum collection and herbaria should be generated by all taxonomists in their monographs. A voucher is made, used as a permanent record to connect the DNA barcode with a particular species of plant, fungus or animal.
- 2. Unknown sample' barcode sequence is matched against the barcode library for identification- Once the barcode library is generated. Then, unidentified sample's DNA barcode is compared to the known sample's barcode using some type of matching algorithm. A matching tool, Basic Local Alignment Search Tool is provided by Genbank that search the relation between a questioned sequence and a sequence library.

The process is ended with the identification of unknown sample. While for the answering of biological questions, barcodes can also be used as tools (Kress, 3).

Applications of DNA Barcoding





- 1. Agriculture Pest Control: The identification of pest in any stage of life can be done by DNA barcoding and make easy to control them. The management of fruit flies is contributed by barcoding with the identification and stop them at border.
- 2. Disease Vectors Identification: The identification of vector species that can cause severe infective diseases to organism and curing by DNA barcoding.
- **3. Sustaining Natural Resources**: Illegal trade of products made of natural resources (Hardwood Trees) are monitored by natural resource managers using DNA barcoding.
- 4. Endangered Species Protection: DNA barcoding is used by law enforcement to protect the endangered species.
- 5. Monitoring Water Quality: The health of species, living in the lakes, ponds and streams can be measured by DNA barcoding. It can improve the determination of quality and create better policies to ensure safe supply of drinking water.
- 6. Natural health Products Authentication
- 7. Identification of plant leaves when flowers or fruits are not present
- 8. Medicinal Plant Identification (Kaur, 2015).

Review of Literature

Sahare and Srinivasu (2012), DNA barcodes allows the identification of species from even a small

processed material. For the identification of medicinal plants at the molecular level ITS region barcode sequencing has been used. Eco RV with Each of the plant specific ITS region gives unique digestion that can be again used for identification.

Ghosh, Mahadani and Sharma (2013) researched on Rauvolfioideae (family of Apocynaceae) medicinal plant and found matK sequence information (DNA barcode) is helpful in correcting the species identification for medicinal plants of Rauvolfioideae and also help in providing diagnostics for the identification of mal species forensics in herbal formulation.

Techen, et al. (2013), the increasing demand for herbal remedies, authentication of medical plant material is important. For easy identification, it is important to provide a sole, extensive database with DNA data. The Barcode of Life Plant Working Group recommends the genomic regions rbcL, matK for barcoding, but other genomic regions could be more useful for medicinal material identification. Furthermore, depending on the material analyzed, one or the combination of up to three genomic regions was necessary to provide the required information for identification.

According to Duan *et al.* (2014) DNA barcoding have some problems in the identification of plant species as universal DNA barcode in plants is very difficult to obtain because of the widespread hybridization in the plants. Some DNA barcodes have poor universality. The barcode is based on traditional taxonomy due to which the accuracy of experimental materials is critical. Hence, barcode and the combination of barcode's ability for identification should be compared and evaluated by plant taxonomy, molecular biology, bioinformatics and other methods.

Aziz, Ahmad and Naim (2015) studied identification of medicinal plants. He used three DNA Barcode for his study ITS2, rpoC1, and trnH-psbA and gave a conclusion that trnH-psbA is the best marker for the differentiating 12 medicinal plant used by Malay traditional healers. Before, this study there was no record of these plants species in the Genbank.

Hubert and Hanner (2015) proposed that the linkage of DNA world with traditional approach of taxonomy, DNA barcoding has been settled as new standard for data quality, accessibility and reproducibility which make the use of DNA sequences in others field of biology more sustainable. The DNA sequencing is not only helpful in objective methods for species delineation as well as new tools for species identification but significantly challenged the manner of collection and creating biodiversity knowledge publicly available and paved the way more sustainable practices in taxonomy.

Raja *et al.* (2017) tried to find out identification of fungal samples by ITS barcoding marker and concluded that dietary companies can validate the accuracy of ingredient in dietary supplements. Barcoding methods can give the guaranty of industry's product reliability, consumer safety and product integrity. In their study, they showed the pros and cons of ITS barcode marker in the fungi.

Enan *et al.* (2017) compared the herbarium plant with the fresh plants using matK, rbcL, and rpoC1 barcode marker. They concluded that fresh samples are better amplified compare to herbarium samples, and neither matK nor rpoC1 are sufficient to identify the plant species while rbcL regions has real prospective to distinguish the plant species into suitable family and genus.

Braukmann *et al.* (2017) studied on Canadian Flora. The efficiency of DNA barcoding was tested across a diverse set of communities in Canadian flora. Three standard barcode markers (rbcL, matK, ITS2) were used in this study and concluded as three barcodes are efficient separately (>90%). matK showed the high resolution while ITS2 showed slightly lower performance. according this paper, ITS2 has two major advantages short length makes it fit for HTS based application and it can be recovered from diverse taxa as well as form vascular plants and fungi.

Future Perspective of DNA barcoding

A new perspective of DNA barcoding is "purposedriven barcode" which is suitable for multi levels such as identification of living organisms, reconstructing community phylogenies, detecting environmental biodiversity information and exploring ecological network structure. Mega phylogenies in face of the post genomic era would be generated by developing new integrative sequencing strategies. For land integrating genetic, morphological and environmental information, intelligent identification systems or online server platforms will established to make DNA based plant identification more precise, convenient and interesting. For commercial authentication and endangered plant taxa against the illegal international trade, national-level DNA barcode sequence libraries of economically valuable tree species will be constructed (Pei, Chen and Kress, 2017).

Conclusion

DNA barcoding is fast and accurate method for the species identification in which different types of barcode are used. But these different barcodes are not

applicable to all plant species as the large number of plant species are present in the universe. One barcode works for the many types of species so it is difficult to determine the species on the basis of one barcode. It gives the reliability and integrity of the products and ensure the safety of consumer as in the dietary products. From this review study, we concluded in the end as more than one genomic region should be examined for the identification of species.

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- 1. Academic Journal of Accounting and Finance (AJAF)
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- 3. Academic Journal of Anthropological Studies (AJASt)
- 4. Academic Journal of Applied Engineering (AJAE)
- 5. Academic Journal of Archaeological Studies (AJALS)
- 6. Academic Journal of Arts and Humanities (AJAAH)
- 7. Academic Journal of Astrophysics and Planets (AJAP)
- 8. Academic Journal of Bioinformatics (AJBI)
- 9. Academic Journal of Biotechnological Research (AJBR)
- 10. Academic Journal of Botanical Sciences (AJBS)
- 11. Academic Journal of Chemical Sciences (AJChS)
- 12. Academic Journal of Computer Sciences (AJCoS)
- 13. Academic Journal of Dance and Music (AJDM)
- 14. Academic Journal of Earth Sciences and Geological Studies (AJESGS)
- 15. Academic Journal of Economic and Finance (AJEF)
- 16. Academic Journal of Educational Sciences (AJEdS)
- 17. Academic Journal of Environmental Sciences (AJES)
- 18. Academic Journal of Forensic Sciences (AJFSc)
- 19. Academic Journal of Forestry Sciences (AJFS)
- 20. Academic Journal of Geographical Studies (AJGS)
- 21. Academic Journal of Historical Studies (AJHS)
- 22. Academic Journal of Home Science and Food Technology (AJHSFT)
- 23. Academic Journal of Information Security (AJIS)
- 24. Academic Journal of Law and Judiciary (AJLJ)
- 25. Academic Journal of Library and Information Studies (AJLIS)
- 26. Academic Journal of Life Science (AJLS)
- 27. Academic Journal of Literature and Language (AJLL)
- 28. Academic Journal of Management Studies (AJMSt)
- 29. Academic Journal of Material Sciences (AJMSc)
- 30. Academic Journal of Mathematical Sciences (AJMMS)
- 31. Academic Journal of Medical and Health Care Sciences (AJMHCS)
- 32. Academic Journal of Microbiological Studies (AJMBS)
- 33. Academic Journal of Modern Applied Sciences (AJMAS)
- 34. Academic Journal of Nanotechnology (AJNT)
- 35. Academic Journal of Nursing and Midwifery Studies (AJNMS)
- 36. Academic Journal of Ocean Sciences (AJOS)
- 37. Academic Journal of Pharmaceutical Sciences (AJPMS)
- 38. Academic Journal of Physical Sciences (AJPSc)
- 39. Academic Journal of Psychological Studies (AJPSt)
- 40. Academic Journal of Social Sciences (AJSS)
- 41. Academic Journal of Sports and Physical Education (AJSPE)
- 42. Academic Journal of Veterinary Sciences (AJVS)
- 43. Academic Journal of Zoological Sciences (AJZS)





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