2. Review of Literature
2.1. Regeneration:

Regeneration or tissue repair is an interesting phenomenon in biology; it is fascinating to observe the regeneration ability in different animals. Lazzaro Spallanzani (1768) when he reported about the regeneration ability of decapitated snails to regain their functional head again in response to amputation. Numerous scientists, public and philosophers are interested to scoured their gardens in an attempt to replicate this fascinating experiment (Odelberg, 2004). The search of the animal in the context of regeneration ability reveals the identification of many species to have this remarkable ability. Salamanders have the ability to regenerate their lost limbs, tails, retina, heart, brain again which includes even the spinal cord (Carlson, 2007; Brockes and Kumar, 2005; Brockes and Kumar, 2002; Parish et al., 2007; Ferretti et al., 2003; Singh et al., 2010; Tanaka and Reddien, 2011). For an instance it was reported that planarians have a remarkable property to regenerate their entire body from a small portion of their fragments (Morgan, 1898). Urodele amphibians have an unmatched striking ability to regenerate their upper and lower jaw, lens, retina, limb, tail, spinal cord and intestine (Brockes and Kumar, 2005). The knowledge gathered in the study of regeneration across different species in the animal kingdom implies the sort of regeneration ability widespread among them. Even though the vast knowledge is available about the regeneration across the animal kingdom, the realistic regeneration events in terms of cellular and in molecular aspects are still in the early stages. The genetic and molecular tools are addressing this event in a rapid fashion nowadays. The knowledge obtained in the field of regeneration helps in better understanding the process and has a practical impact in the field of medicine in a near future.

The Regeneration potential varies among different species and it also depends on the extent of damage. Among the different animals known for their regeneration ability, Annelids
are more peculiar because this highly organized animals has the ability to reform the complete structures. In higher megadriles oligochaetes, sporadic cases occur whose powers of regeneration are nearly equal to those of the microdrilous genera as the earthworm *Perionyx excavatus*. But in general, among the earthworm only anterior levels are able to regenerate a head, and this ability cases in the neighbourhood of the 14th to 20th segments.

Regeneration is supported by the nervous system, in responding to tissue damage they are triggered to release the trophic factors that aids in the functional recovery from damages by mediating repair mechanism in peripheral tissues and activate cell proliferation at the site of the wound (Herlant-Meewis, 1964). Besides the nervous system, Growth factors and hormones can also mediate regeneration processes they are likely synthesized in response to injuries from different tissues like a body wall or alimentary canal. In addition, it was reported that earthworm VNC (Ventral Nerve Cord) plays a vital role in regeneration perhaps it also has a remarkable capability to regenerate itself after injury (Herlant-Meewis, 1964; Lubics et al., 2002). One of the interesting aspects in the nervous system regeneration is that it requires reemployment of mechanisms used during brain development and PACAP (Pituitary Adenylate Cyclase-Activating Polypeptide) might be one such factor (Waschek, 2002). It has been reported that PACAP has the evolutionary conserved contribution in several mechanisms of vertebrate and invertebrate nervous regeneration (Kammermeier and Reichert, 2001).

When comparing the different forms of life it has been outlined that earthworms are able to regenerate their entire anterior portion which includes central nerve system, heart and clitellum with highest evolutionary value. This beneficiary aspect along with the availability and ease of culture and handling makes *P. excavatus* (earthworm) an excellent material for studies of regeneration mechanisms (Gates, 1927). The study carried out in *P. excavatus*
during head and tail regeneration helps to identify the expression of full length of three labial genes (Pex-lab01, Pex-lab02 and Pex-lab03) (Cho et al., 2009).

2.2. Types of Regeneration:

Naturally animals are exhibiting various forms of Regeneration ability. Some of the living organism is able to replace the worn out parts, and even some are able to repair or renew damaged or lost parts of the body or to reconstitute the whole body from a small fragment of an organism. There are three types of regeneration commonly available. They are.

1. Physiological regeneration: This type of regeneration is going on continuously to compensate the loss of cells naturally. The replacement of such cells is described by the terminology, physiological regeneration. E.g., Replacement of R.B.C's, renewal of gut and hair follicle epithelial cells.

2. Reparative regeneration: This type of regeneration occurs when an organ or tissue are detached by external causes, in that cases the replacement of lost parts or repair of damaged body organs occurs parallel. E.g., Regeneration of limbs in salamander.

3. Autotomy: Autotomy is nothing but the self amputation of body tissues in the condition of injury or at the time of external attack. E.g., Crabs detach their own leg to escape from their natural enemy.

2.3. Earthworms:

In the taxonomical world earthworms are classified as animals belonging to the order oligochaeta, Class Chaetopoda, Phylum Annelida, which have originated about 600 million years ago, during the pre-Cambrian era (Piearce et al., 1990). Earthworms were referred by Aristotle as “the intestines of the earth and the restoring agents of soil fertility” (Shipley,
A high density of earthworm in a particular place indicates the presence of their associated bacteria, viruses, fungi, insects, spiders and other organisms and thus it specifies the healthy soil (Lachnicht and Hendrix, 2001). Soil fertility test mostly relies on Earthworms and it was the common assay since they represent a major group in the terrestrial environments (Weeks and Svendsen, 1996). Earthworm directly helpful for soil fertility by their casts that helps to enrich the soil rich in phosphorous, magnesium, calcium and nitrogen. Earthworms help to increase the production of plants in agricultural areas (Edwards and Bohlen, 1996). All over the world there are about 3000 species of earthworms reported and out of that about 384 species are reported in India (Julka, 1986). The soil habitat earthworm has an average age ranges from 3 to 7 years and it usually depends upon the type of species and the ecological situation (Edwards and Lofty, 1972; Gunathilagraj, 1996).

It has been reported that in recent years, organic wastes are more and more accumulated due to the increasing domestic, agricultural and industrial sources that result in various environmental problems. Organic wastes from sewage sludges and solids from wastewater are treated with microbial composting through the help of earthworm activity and it proved to be successful (Neuhauser et al., 1988; Domínguez et al., 2000). Similarly materials from breweries (Butt, 1993), paper wastes (Butt 1993; Elvira et al., 1996), urban food and garden residues, animal wastes (Edwards and Fletcher, 1988; Elvira et al., 1996; Domínguez and Edwards, 1997), as well as horticulture residues from processed potatoes, dead plants and the brewery and from mushroom industries are easily decomposed using earthworm aided microbial composting.

2.4. Types of earthworm:

Earthworms are generally classified as saprophages but based on their feeding and burrowing habits they are classified into detrivores and geophages (Lee, 1985). In general
majority of the earthworm are omnivores however *Agastrodilus*, a genus of the earthworms from the Ivory coast of Africa has been reported as carnivorous. They feed upon other earthworms of the family Eudrilidae (Levelle and Satchell, 1983).

1. **Detrivores:** This category of earthworm mainly depends on plant based products and it found near the surface of the soil. They are mainly feeds on plant litters, dead roots and other plant debris or on mammalian dung. These worms are called “humus farmers”. *Eudrilus eugeniae, Perionyx excavatus, Eisenia foetida, Lampito mauriti, Octochaetona serrata*, are few examples of detrivores earthworms (Ismail, 1997).

2. **Geophages:** These types of worms are available beneath the surface of the soil and they mainly ingest on large quantities of organic rich soil and comprise the endogeic earthworms, *Metaphire postruma* and *Octochaetoma thurstoni* are two common examples of geophages.

**2.5. Earthworm as medicine:**

Traditionally earthworms are used worldwide among the indigenous people to extract the medicinal property compounds and this type of practice is more prevalent in Asia (Ranganathan, 2006). The earthworm, which has stasis removal and wound-healing functions, is broadly used as an herbal medicine in China (Cooper, 2005). Earthworms are extensively used as a medicine for various remedies. Earthworms have long been used as materials to make drugs for antipyretic and diuretic purposes in traditional Chinese medicine. It has a role in anti-inflammatory, analgesic and in antipyretic effects. It seems to have anticancer effects also. It has potent antimicrobial activity and anticoagulant activity. The antimicrobial effect of *E. eugeniae* was well studied and reported by Shobha and Kale, 2007. It was reported that high polyphenolic content in earthworm tissue is responsible for the anti-
inflammatory activity along with antioxidant properties (Balamurugan et al., 2007). A crude form of extracts from earthworm has a thrombolytic effect which significantly promotes blood circulation to remove stasis (Zhang and Wang, 1992). Lumbrokinase, an enzyme extracted from the earthworm has been used to treat stroke and cardiovascular diseases (Jin et al., 2000). Lumbrokinase is a group of proteolytic enzymes (Cooper et al., 2004) that include a plasminogen activator and plasmin. It directly dissolves fibrin and also activate plasminogen (Hu and Fu, 1997). In healthy human volunteers, oral-administered of earthworm powder increase the levels of tPA and fibrinolytic activity (Mihara et al., 1992).

2.6. Earthworm Regeneration:

The recent advances in the field of regeneration and stem cell biology raising lots of outlook in a near future with human regenerative medicine and to attain this aim, the research are accelerating in a wide range of model systems that utilize different regenerative strategies (Poss, 2010). Among the different animal models for regeneration studies annelids exhibits an impressive form of regenerative abilities. But this ability varies widely. It was also reported that some annelids exhibit a similar regeneration pattern like that of planarians by dedifferentiation and redifferentiation, without the participation of totipotent stem cells (Thouveny and Tassava, 1988). Among the annelid worms many are limited in their ability to regenerate anterior body parts, whereas posterior segment regeneration has been frequently common. Charles Bonnet was the first to discover the regeneration ability of earthworms and he published his findings in 1744. Earthworms have a wide array of regeneration ability but this ability differs among different species and it also depends on the extent of the damage. In most of the worms the amputated rear half of the worm will die, but the front half may live and replace the lost half of its body. The anterior or front end must be adequate to contain the head, clitellum and at least 10 segments on the posterior side of clitellum and this makes up
almost half the length of the worm. The regenerating posterior segments will appear in light brown in colour due to lack of pigmentation (Gates, 1927) and looks slightly smaller in diameter than the original segments. In molecular level, different genes are up regulated or down regulated that might activate the stem cell for inducing regeneration.

In an overall earthworm is valuable unique model system to explore the mechanism of regeneration in that, the regeneration takes a relatively short period of time. In order to regenerate its head again it has to regenerate many organs associated with them like mouth, brain, heart, seminal vesicle testis and so on. Similarly when it regenerates its tails it have to regenerate its intestine, chloragogue tissue (hepatoid tissue of earthworm), nephridia, setae, septa and so on. Another advantage in using earthworm as a model system is that it can regenerate in a bidirectional way. It is reported that among the anterior portion regenerating model system which could capable to regenerate brain, heart and other vital organs, earthworm is the highest evolutionary form.

2.7. *Eudrilus eugeniae*:

The earthworm species, *Eudrilus eugeniae* commonly referred to as the West African night crawler and it commonly widespread in warm regions of the world. Even though the *E.eugeniae* are scattered around the world it mostly accumulated in West African regions (Shagoti, 1985; Segun, 1998). It is well adapted in the temperature range of 25°C to 30°C but grow best at 30°C (Viljoen and Reinecke, 1992). The earthworm starts to attain its maximum weight, length and number of segments in about 15 to 20 weeks (Rodriguez and Lapeire, 1992). The size of the worms mainly depends on the growing habitat and it ranges from 10cm in length to huge specimens of over 12cm (Segun, 1998). The worm appears in purple sheen and the posterior segments evenly taper to a point (Blackburn, 1989).
The mature worm of *E. eugeniae* on an average has about 80–100 segments (Oboh *et al.*, 2007). Most of the vital organs of this earthworm are located within the first 13 segments and it comprises mouth, simple brain, four pairs of pseudo-heart, testis, seminal vesicle, rudimentary ovary, oviduct and accessory glands of the ovary. Just behind the anterior part of the worm from 13–18 segments, a thick cylindrical collar-like structure called the clitellum are present which plays a major role in reproduction. This species of the earthworm have high reproductive potential and its total life span is 1-3 years (Edwards and Lofty, 1969). The posterior part has some vital organs such as a pair of prostate glands, intestine and the anus. All the internal organs are in the coelomic fluid, which also has different types of cells called coelomic cells. They have the property of autofluorescence (Cholewa *et al.*, 2006). The source of the fluorescence is mainly riboflavin. Autofluorescence has been reported in the body setae too (McManus, 2007).

The segmented worm *E. eugeniae* is the common type of earthworms used for vermicomposting in tropical and sub-tropical countries (Somniyam and Suwanwaree, 2009). Vermicomposting is nothing but a process carried out by earthworm which includes ingestion, digestion and absorption of organic waste followed by a breakdown of the complex organic matter into simpler form with the help of bacteria and to excretion of castings through the worm’s metabolic system, which enhance the levels of plant-nutrients of organic waste during their biological activities (Chaudhuri *et al.*, 2009). In some part of the world *E. eugeniae* to be used as fish bait (Dominguez *et al.*, 2001).

2.8. *Perionyx excavatus*:

The earthworm *P. excavatus* is commonly found over a large area of tropical Asia (Gates, 1972). The epigeic species usually live in organic wastes and in the places where there is high moisture contents. The life cycle and their ability to break down organic wastes
have been well documented by different groups (Kale et al., 1982; Reinecke and Hallatt, 1989; Reinecke et al., 1992; Hallat et al., 1992).

The earthworm, *P. excavatus* attain a maximum length up to 130 mm and to a diameter of 5 mm. Mature worms can be available in large numbers in all over the years. And one peculiar advantage of them is it easily adjusted to the lab condition and it can have a low mortality rate after an operation or injury. It has the enormous regeneration ability, even the multiple amputations of post-clitellum segments can generate many individual worms but the pre-clitellated portion from segment 1-20 does not possess the regeneration ability (Gates, 1927).

*E. eugeniae* and *P. excavatus* have high potential features like quick life cycle, fast regeneration capacity, highly reproductive and easy to maintain. So it is very suitable for research purpose when compared to other worms.

2.9. Research activities using earthworm:

Otitoloju, (2005) reported that the earthworms *E. eugeniae* can be used as a stress indicator. The exposure of the animal in sublethal concentrations of crude oil has an effect on their weight, haemocyte counts and have changes in clitellum structure. The significant results act as a stress indicator to identify and assess recovery of crude oil contaminated ecosystems.

Balamurugan et al., (2008) assess and monitor the hepato protective potential of earthworm extract (*Lambito mauritti*) against paracetamol induced liver injury in Wistar albino rat, in comparison with the standard hepatoprotective drug silyramin.

The interesting results have been reported by Takahashi et al., (1996) that the J-chain gene is expressed in invertebrates (Mollusca, Annelida, Arthropoda, Echinodermata, and
Holothuroidea), as well as in representative vertebrates (Mammalia, Teleostei, Amphibia). Furthermore it was evaluated that J-chain cDNA from the earthworm has a high degree of homology (68-76%) to human, mouse, and bovine J chains.

The anticancer studies of earthworm extracts were carried out by Yan et al., (2011) and he reported that the high dose of earthworm extract decreased the cell proliferation and neuroblast differentiation.

Chang et al., (2011) reported the roles of MAPK pathways at the time of neuronal regeneration of earthworm. Schwann cell migration is critical for the regeneration of injured nerves. They have concluded through their result that the MAPKs signalling pathway of Schwann cells is necessary for the migration of Schwann cell inorder to carry out nerve regeneration.

Giraddi et al., (2008) studied the reproductive biology between two species of earthworms, E.eugeniae and P.excavatus. It was observed that the E.eugeniae produced 6.75 cocoons/week as against 2.63 cocoons/week seen in P.excavatus. Number of neonates per cocoon was higher (2.71) in E.eugeniae, whereas it was 0.81 in P.excavatus. They also observed that seasonal variation has no effect on incubation period as well as neonate numbers /cocoon, for both the species. In case of hatching percentage it was observed that both the worms are influenced by seasonal changes. The overall results implies that the earthworm, E.eugeniae is the prolific breeder when compared to P.excavatus.

Brise and Bittner, (1981) documented the regenerative ability of the septate medial giant axon (MGA) and the septate lateral giant axons (LGAs) of the earthworm ventral nerve cord (VNC) and from their study they concluded that the mechanism of giant axon regeneration is rather similar following transection or cell body ablation; that is, neuronal
processes arising from a giant axon grow across the lesion site and make functional connections only with the appropriate giant axon on the opposite side of the lesion.

Jiang et al., (1990) reported that the yellow mucus fluid isolated from *Lumbricus terrestris* has alarm properties for conspecifics and chemo attractive properties, for garter snakes.

Gupta, (2000) used the earthworm *Metaphire posthuma* as a biomarker for assessing the toxic potential of cadmium that are incorporated into the soil by environmental or human activities. The retention period of neutral red in the coelomocytes of earthworm was used as a biomarker for this work. This study highlights that the presence of cadmium caused damage to the lysosomes of Coelomocytes.

Jing Zhao et al., (2007) had found out that the earthworm, *Eisenia fetida* proteases have the function of fibrinolysis and he isolated and purified the protease-II (EfP-II) and protease-III- from *Eisenia fetida*. The study shows that the enzyme activity was mainly detected around the clitellum segments of the worm.

Cho et al., (2009) analyzed and construct a cDNA library for the earthworm *Perionyx excavatus* at the time of anterior regeneration. Their group analyzed 1,159 expressed sequence tags (ESTs) derived from cDNA library. The study reveals that 53.7% of ESTs showed significant similarity to known genes, 57.2% were sequenced only once, 16.2% were sequenced more than five times. The ESTs used in this study provide a resource for future research in earthworm regeneration.

It has been reported that the earthworm has a remarkable property to accumulate the soil metals in their tissues and it can be effectively used to estimates the extend of metal pollution in that particular location (Suthar et al., 2008).
2.10. Stem cells:

The organization of human body comprises of 200 different cells that compose to form different tissue and organs. They are required for the viability and reproduction. The stem cell concept arises when some tissues like intestinal epithelium, skin and blood have short life span and unable to self-renew that led to a concept that this cells are maintained by high renewal capacity cells called stem cells (Lajtha, 1979). In other terms stem cells are described as undifferentiated cells found in all multicellular organisms, which have the ability to divide and differentiate into a diverse group of cell types and can self-renew to produce more stem cells over a lifetime. Research into stem cell grew out of findings by McCulloch and Till at the University of Toronto in the 1960s. The ability of the stem cell to differentiate into different types of cells is known as potency (Scholer, 2004).

The presence of the stem cells is rare in most tissues and it is difficult to identify and purify them. Isolation and identification of somatic stem cell have success in a few instances. For example, haematopoietic stem cells have been isolated from mice and humans (Spangrude et al., 1988; Morrison and Weissman, 1994; Baum et al., 1992; Osawa et al., 1996), and have been shown to be responsible for the generation and regeneration of the blood-forming and immune (haematolymphoid) systems. Stem cells have the ability to form a variety of organs and it might have the therapeutic uses in the near future, but HSCs the vital elements in bone-marrow transplantation have already been used extensively in therapeutic settings (Akashi and Weissman, 2001). Developing tooth bud of the mandibular third molar is the richest source for adult mesenchymal stem cell (Huang et al., 2009).
2.11. Totipotent Stem Cells:

Totipotent stem cells have the remarkable property to form any kinds of cells of the body (Mitalipov and Wolf, 2009). Totipotent stem cells develop at the time point of reproduction when male and female gametes fuse during fertilization to form a zygote. The zygote is totipotent in nature and it has the unlimited ability to form any type of cell. As the zygote continues to divide and mature, its cells develop into more specialized cells called pluripotent stem cells.

2.12. Pluripotent Stem Cells:

Pluripotent stem cells have the ability to differentiate into many types of cell in the body but it cannot have the ability to generate the complete organism (Evans and Kaufman, 1981; Martin, 1981). After the embryonic development stage gets completed their pluripotency is lost and they can only become certain types of cells. Specialization in pluripotent stem cells is minimal and therefore they can develop into almost any type of cell. Embryonic stem cells are isolated from the inner cell mass of the blastocyst. Embryonic stem cells and fetal stem cells are two types of pluripotent cells.

2.13. Induced Pluripotent Stem Cells (iPS Cells):

Recent studies in the field of stem cell biology make the possibilities of converting the somatic cell by manipulating to exhibit the properties of embryonic stem cells. They are genetically modified adult somatic cells that are manipulated in the laboratory to resemble the characteristics of embryonic stem cells. The altered or reprogrammed cells exhibit the similar characteristic function of embryonic stem cells and it has the capability to differentiate into many different cell types in the lab and it is possible to produce Disease-specific induced pluripotent stem cells (Park et al., 2008). Even though the induced pluripotent stem cells
have similar properties like that of embryonic stem cell due to the expression of some similar genes that are expressed normally in embryonic stem cells, they are not the exact duplicates of embryonic stem cells.

**2.14. Multipotent Stem Cells:**

These types of stem cells have the capability to differentiate into multiple types of cells but with limited cell types. Multipotent stem cells typically have the ability to develop into a particular cell type or group (Lajtha, 1979). For example, bone marrow stem cells can have the potential to produce any type of blood cells. But the restriction in the multipotent stem cells is that it cannot able to form other cell types eg., bone marrow cells do not produce heart cells but it can differentiate to produce all cell types of the blood. Adult stem cells and umbilical cord stem cells are examples of multipotent cells.

**2.15. Mesenchymal Stem Cells:**

Mesenchymal stem cells (MSCs) are multipotent property having adult stem cells occur in the bone marrow. Through their multipotent ability they are able to differentiate to form osteoblasts, adipocytes, chondrocytes, myocytes and neurons. Even though the MSCs have the ability to differentiate into several types of specialized cells related to but it cannot have the ability to differentiate into blood cells. The morphology of mesenchymal stem cells looks like long thin cell bodies and with a large nucleus. As with other stem cell types, MSCs have a high capacity for self renewal while maintaining multipotency.

**2.16. Oligopotent Stem Cells:**

Oligopotent stem cells can only have a limited potential of differentiation and they are able to form only a few cells, such as lymphoid or myeloid stem cells (Scholer, 2007). For example lymphoid stem cell cannot able to differentiate into any other type of blood cell as
bone marrow stem cells can. They only have very limited ability to give rise to only blood
cells of the lymphatic system, such as T cells.

2.17. Unipotent Stem Cells:

These types of stem cells have only restricted form of differentiation. They are only
able to differentiate into the only single type of cells or tissue but this type of stem cells has
unlimited reproductive capabilities. Examples of Unipotent stem cells are muscle stem cells
and skin stem cells. The unipotent stem cells are usually derived from the multipotent stem
cells and formed in adult tissue. These types of stem cells can readily undergo cell division to
replace damaged cells.

2.18. Stem Cell Niche:

Stem cells are emerging as one of the fundamental underpinnings of tissue biology. They allow blood, bone, gametes, epithelia, nervous system, muscle, and myriad other tissues
to be replenished by fresh cells throughout life. Additional stem cells lie dormant, but can be
activated at particular life cycle stages, or following injury. These potent agents are
controlled within restricted tissue microenvironments known as “niches.” Until recently,
niches were a theoretical concept strongly supported by the observation that transplanted
stem cells survive and grow only in particular tissue locations. The number of such sites
could be saturated, after which transferring additional stem cells provided little or no further
engraftment. However, in recent years it has become possible to identify stem cells and
niches with increasing precision. In this review we summarize progress in delineating stem
cells and their niches, as well as in discovering the mechanisms that control stem cell
function. Finally, Morrison and Spradling, (2008) examine how niches change with age and
contribute to cancer and tissue aging.
Stem cell niches are dynamic microenvironments that balance stem cell activity to maintain tissue homeostasis and repair throughout the lifetime of an organism. The development of strategies to monitor and perturb niche components has provided insight into the responsive nature of the niche and offers a framework to uncover how disruption of normal stem cell niche function may contribute to aging and disease onset and progression. Additional work in the identification of genetic factors that regulate the formation, activity, and size of stem cell niches will facilitate the incorporation of the niche into stem cell-based therapies and regenerative medicine. (Justin and Leanne, 2010)

Niches are local tissue microenvironments that maintain and regulate stem cells. Long-predicted from mammalian studies, these structures have recently been characterized within several invertebrate tissues using methods that reliably identify individual stem cells and their functional requirements. Although similar single-cell resolution has usually not been achieved in mammalian tissues, principles likely to govern the behavior of niches in diverse organisms are emerging. Considerable progress has been made in elucidating how the microenvironment promotes stem cell maintenance. Mechanisms of stem cell maintenance are key to the regulation of homeostasis and likely contribute to aging and tumorigenesis when altered during adulthood (Morrison and Spradling, 2008).

2.19. Prostate cancer:

Cancer is a life-threatening disease and one that develops in the prostate gland in the male reproductive system is called prostate cancer. Prostate cancer (PCa) is the most predominant type of cancer among the male population. It accounts for the second most male cancer-related deaths among the Western world population (Thorne et al., 2011). Latest statistics analysis carried out by Prostate Cancer Foundation of Australia found out that, the annual die of men due to PCa is almost equal to that of women die from breast cancer.
Another statistical analysis implies that in Australia itself around 1,20,000 men are living with this severe disease as every year 20,000 new cases are emerging and approximately 3,300 will die each year. In a Global view of this problem, it was stated that one of those are died per four minutes due to PCa. The pace of diagnosis of PCa related issues are increased dramatically over the past decades due to the awareness among the older age groups and by the counterpart introduction of the prostate specific antigen (PSA) test (Bangma et al., 2007). Currently the available most common form of diagnostic tools for PCa includes Digital Rectal Examination (DRE), PSA and subsequent biopsies for histopathological staging (Assinder and Nicholson, 2007). However, all this available diagnostic tool has its own limitations when practically approach it, and that limitation pays way to over-treatment of low-risk patients (Schroder et al., 2009), unnecessary biopsies and non-essential radical prostatectomies (Moyer, 2012). Therefore there is a lot more need in this area to accurately evaluate the present biomarkers and to come with the novel biomarkers in research settings to maximize clinical benefit.

2.20. Biomarkers:

A biomarker is defined as a “characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacological responses to a therapeutic intervention” (Atkinson et al., 2001). It may be related to blood test, radiographic measurements or it is planned to guide the patient management programmes. The biomarkers should meet any one of the following purposes whether it may serve as prognostic, predictive, or surrogate in nature, or it can posses multiple properties of any of these. A prognostic biomarker offers an evidence about a patient’s ultimate outcome of a disease independent of a given therapy, whereas a predictive biomarker estimates the likelihood of response to a specific therapy in a specific context (Dancey et al., 2010). The
well suited example for predictive biomarker is that HER2 oncogene which is overexpression in breast cancer patient and it serves as a prognostic and also to predict benefit with trastuzumab treatment (Slamon et al., 2001). A surrogate biomarker goes further and is able to substitute as an intermediate for a clinically meaningful end point (Prentice, 1989). To be effectively used as a surrogacy biomarker it should fulfil or must satisfy several key statistical criteria (Prentice, 1989; Buyse et al., 2000; Freedman et al., 1992; Buyse et al., 2010) and also it should be validated over with many trials with a variety of mechanistically distinct agents (Prentice, 1989; Buyse et al., 2000).

2.21. Biomarker Challenge in Prostate Cancer:

The PubMed Search related to “prostate cancer” AND (“biomarker” OR “marker”) yield only 3016 hits three years back (accessed 8/8/2010). But the research activities and the number of articles published related to PCa biomarkers has increased steadily over the current years. Despite a lot of biomarkers reported to be promising for PCa, the Prostate Specific Antigen (PSA) is regularly used by urologists. But many of the other biomarkers do not work upto the extent of their promise due to many analytical and regulatory barriers (Bensalah et al., 2007; Shariat et al., 2004). The limit of diagnostic lack may be due to non availability of standard reference materials for the assay, difficulty with the assay set-up, lack of assurance in reproducibility and inaccuracy, lack of clear guidelines for good manufacturing/laboratory practice and the cost and effort needed to accumulate clinical data in different Institutional Review Board-approved prospective trials, patent issues, assay standardization, validation, testing, and regulatory approval. Other than this Research Network developed, five phase research guidelines for the systematic discovery and validation of biomarkers (Bensalah et al., 2007; Shariat et al., 2004; Bensalah et al., 2007a).
Any newly designed biomarkers before it reach to the community benefit, it needs to address four issues: “better, easier, faster and cheaper” (Shariat et al., 2004).

Identifying the effective biomarkers requires, understand of the problems and to know the key cancer-related pathways that are essential for developing the new and improved diagnostic and predictive tools. The identification and to develop the tumor-specific biomarkers has been a great challenge and it requires a proteomic approach that contributes significantly to the identification of serum biomarkers for PCa (Goo and Goodlett, 2010). Despite technological advances (Nedelkov, 2012), serum proteomics still struggle with identifying the effective diagnostic tool due to the challenges in identifying wide range of protein concentrations, difficulty in tracing out the low-abundance proteins due to the masking effects of high-abundance proteins (Wu and Yates, 2003), elevated levels of salts concentration and other interfering compounds, extreme variations related to different individuals and due to lack of reproducibility in the results which significantly turn the attention to other possible biomarkers.

Biological specimens that are considering for PCa research include blood, urine, semen and prostate tissue. Each specimen groups have its own merit and demerit associated with that and that may affect clinical validation of biomarkers and adoption for routine testing. For instance Human plasma has large groups of protein arrays that might serve as potential markers for PCa diagnosis and prognosis. Another easily available and non-invasive nature source is Urine that has become a popular source for proteomic biomarker discovery. Some markers available in urine source can even used to distinguish between healthy BPH (Benign Prostatic Hypertrophy) and malignant PCa. Semen can also be used to analyzing prostate biomarkers and it also one kind of non-invasive material. One more advantage of using semen is that the Proteins directly from the prostate are easily accessed; however, there
is a compositional variability among patients that poses an issue. Finally, the direct assessing of the prostate tissue, provide a rich source for PCa biomarkers, but it depending on most invasive of sampling sites.

2.22. Prostatic Acid Phosphatase and Prostate Specific Antigen Tests:

Prostatic acid phosphatase (PAP) is a glycoprotein dimer has its origin predominately from the prostate gland and it was initially used as a serum biomarker for the detection of metastatic PCa (Gutman and Gutman, 1938). Unfortunately, PAP has a minimum sensitivity for detecting localized disease (Hernández and Thompson, 2004) and was replaced as a test following the discovery (Hara et al., 1971) and development of the prostate specific antigen (PSA) test. PSA is a serine protease (kallikrein-3) of 33 kDa size protein, that is secreted by the epithelial cells of the prostate gland. In a normal patient with normal prostate gland, PSA is produced and secreted from the prostatic epithelial cells and they passes into the secretory ducts to contribute to the seminal fluid. However, in a patient with PCa, the basal-cell layer get disrupted and that allows PSA to leak into the circulation that resulted in the elevated levels of serum PSA. Even the PSA is enriched in prostate tissue it is not directly indicates the condition of disease (Pinsky et al., 2006). Because the serum PSA level may also goes up in conditions like non-cancer related BPH, prostatitis, diet alterations, medications and environment (Liu et al., 2012). Using a PSA level only it is not possible to distinguish between stages of PCa and it is not possible to identify and distinguish metastatic PCa and that decrease the sensitivity and specificity necessary to make accurate therapeutic decisions (Hessels and Schalken, 2013). Since after the execution of PSA screening among the ageing population, one of the outcome is that it has reduced the average age of PCa diagnosis from 70 to 71 years of age to 67 years of age (Cross et al., 2012). The most prevalent use of PSA in the screening protocol helps to rapid increase in PCa diagnoses in the past two decades. In
recent years the mortality associated with PCa has decreased significantly, but it is uncertain to know whether it is due to the introduction of PSA screening or due to the advances and efficacy of current PCa treatments (Sardana et al., 2008).

Unfortunately, many false positive results are observed while screening with serum PSA test. The set value for PSA test (4 ng/mL) cannot tune enough to detect PCa that are confirmed through prostate biopsy and thus it fails to detect 20% to 40% of cancer cases (Mistry and Cable, 2003; Schroder et al., 2000). It also falsely detects indolent PCa in 40%–50% of cases and they are unnecessarily treated (Lin et al., 2013). Lowering the PSA threshold level below to 4 ng/mL has been an alternative approach to satisfy the current problems of the PSA test. But the problem associated with are increased risk of identifying and that pay way for unnecessary treatment. In addition to this the other problem associated with using a PSA is that when its value are > 4 ng/ml it commonly may be also due to BPH, prostatitis (Haythorn and Ablin, 2011) and rarely by other human malignancies (Bodey et al., 1996).

2.23. Total PSA and its limitations:

PSA is not an efficient marker because it lacks its sensitivity for example in the case of Neoplastic cells it have the ability to produce some extent lower and varying forms of tissue level PSA compared to benign epithelial cells still both the condition results in elevated level of total PSA in the blood (Shariat et al., 2004). The comparative outcome helps to determine that the total PSA should be considered as a marker of BPH which mainly depend on prostate volume, growth, and outcome rather than a reliable marker of PCa (Shariat et al., 2004). Other than this it was claimed that in some aggressive form of prostate cancer even cannot produce PSA and its level vary among the prostate cancer patients individual in this way it affects the interpretation of any single result (Ankerst et al., 2009). The reason for
variation in total PSA level also includes both analytical (depends on sample handling, different laboratory processing methods, assay efficiency and standardization) and biological variation (includes metabolism, renal elimination, medication, physical and sexual activity, size and integrity of the prostate). The fluctuations in results up to 20–30% cases are may be due to the biological variations (Soletormos et al., 2005; Roehrborn et al., 1996). Assay standardization is an another problem that results in high or low estimate of total PSA and total PSAV (Link et al., 2004; Sotelo et al., 2007; Stephan et al., 2006).

The treatment methods have an effect on the total PSA value for instance in case of BSH treatment previously the 5-α-reductase inhibitors were used and it has an effect on the predictive value of total PSA kinetics for tumor progression. Research on these aspects suggests that the 5-α-reductase inhibitors can decrease the PSA level up to ~50% by suppressing the benign components of PSA secretion (Thompson et al., 2006). In an overall the analytical and biological variations in total PSA value between two consecutive measurements are larger and it makes difficult to predict the variations. The calculation of variation as carried out by Nixon et al., reported that the coefficient of variation over 2 weeks implies the significant variation up to 25% (Nixon et al., 1997a; Nixon et al., 1997b). Bunting et al., (2002), reported a critical difference may reach up to 60% over a time period of 1 year. The long-term variability of total PSA levels was recently assessed by Bruun et al., (2005) and it shows the total PSA levels were <2.0 ng/mL at the end of the 8-year observation period (Bruun et al., 2005). They also trace out that the total intra-individual variation of total PSA was much less than it was reported by Bunting et al., (2002) and it shows somewhat higher than the intra-individual variation for either free PSA or percent free PSA. Their results suggest that the concentration of free PSA in the blood may vary less than that of complexed PSA concentration, which is the major contributor to total PSA. One more
explanation is that, free PSA and complexed PSA may vary due to different elimination pathways naturally, and hence it has different elimination rates (Birkenmeier et al., 1999; Bjork et al., 1998; Lilja et al., 1999; Djavan et al., 1999).

The present scenario makes the algorithm complex to predict the cutoff value to define the abnormal total PSA level to be useful in accessing the spectrum of prostate cancer risk. Therefore, it is normally preferred to include and correlate the results of serum total PSA levels with biopsy in order to estimate the overall risk of cancer (Thompson et al., 2004; Thompson et al., 2006; Thompson et al., 2005; Shariat et al., 2008; Kattan, 2005; Kattan et al., 2003; Steyerberg et al., 2007; Nam et al., 2007).