Introduction

“Let food be your medicine and medicine be your food.”

- Hippocrates

The World Health Organization enshrines the highest attainable standard of health as a fundamental right of every human being. The constitution of WHO defines health is a state of complete physical, social and mental wellbeing and not merely the absence of disease or infirmity (WHO, 2009). It is the resource for everyday life and not the object of living and is a positive concept emphasizing social and personal resources as well as physical capabilities. Diet and nutrition plays a prominent role in the promotion and maintenance of good health all through the life.

India faces the dual burden of communicable diseases and chronic Non-Communicable Diseases (NCDs). The dramatic changes in people’s lifestyles driven by health transitions and economic progress are the major contributing factor to the increase in NCDs (Mahal et al, 2009; Popkin, Adair and Ng, 2012; Allender et al, 2010; Arokiasamy and Yadav, 2014). Empirical research has established a strong relationship between economic growth and health (Bloom et al, 2010). Increasing burden of NCDs has not only obvious health implications but also economic and developmental consequences (Mcgarry, 2004, Dwyer and Mitchell, 1999 and Lopez-Casasnovas, Rivera and Curraus, 2005).

According to WHO’s Global status report on Non Communicable Diseases (NCDs) (2014), Non Communicable Disease cause more deaths compared to other causes and deaths and it is projected to increase from 38 million in 2012 to 52 million by 2030. Approximately 42 percent of NCD deaths globally occurred before the age of 70 years in which 48 percent of NCD deaths are in low and middle income countries.
World Health Organization’s global mortality data indicates that chronic disorders like coronary heart disease (Mozaffarian et al., 2012), cancer (Ferlay et al., 2010), stroke (Thrift et al., 2014), chronic obstructive pulmonary disease (Lopez et al., 2006), type 2 diabetes mellitus (Guariguata et al., 2014), renal failure (Levey et al., 2007) and neurodegenerative diseases (Kalaria et al., 2008) are the reason for 38 million deaths in 2009 and approximately more than 62 per cent of deaths worldwide (WHO, 2009).

Renal failure is also called as kidney failure or renal insufficiency. It is a medical condition where the kidneys are unable to filter the metabolic waste products adequately from the blood (Medline, 2012). According to World Health Organization (WHO) Global Burden of Disease project opined that the diseases of the kidney and urinary tract contribute to global burden with approximately 850,000 deaths every year and 115,010,107 disability adjusted life years. CKD is the 12th leading cause of death and 17th cause of disability (WHO, 2006).

The two main classification of kidney disease are Acute Kidney Injury (AKI) and Chronic Kidney Disease (CKD) (Chawla and Kimmel, 2012).

Acute Kidney Injury (AKI) formerly called as Acute Renal Failure (ARF) is a sudden decline in renal function and with reversible increase in nitrogenous waste products in the blood such as blood urea nitrogen and serum creatinine from few hours to weeks (Roy et al, 2013; Hui, Chan and Miu, 2013; Ratanarat et al, 2013; Ricci and Ronco, 2013). Acute Dialysis Quality Initiative (ADQI) was developed with the goal for developing evidence based guidelines for prevention and treatment for AKI. The system of RIFLE (Risk, Injury, and Failure; Loss; End stage kidney disease) was used to classify AKI (Bellomo et al, 2004).

Owing to increasing prevalence of diabetes (Danaei et al, 2011), hypertension (Kearney et al, 2005), obesity (Swinburn et al, 2011), Chronic Kidney Disease (CKD) has become the worldwide public health problem with a substantial economic burden. In western countries, hypertension and diabetes account for over two-third of cases of CKD (Synder and Pendergraph, 2005). In India too, diabetes and hypertension account for 40 to 60 per cent of
chronic kidney disease cases (Rajapurkar et al, 2012). Chronic glomerulonephritis is the second common cause and accounts for sixteen percent next to diabetes and hypertension (First annual report of Indian CKD registry, 2007). Indeed, it has been recently estimated that the age-adjusted incidence rate of ESRD in India to be 229 per million populations (Modi, 2006) and greater than one lakh new patients enter renal replacement programs annually in India.

Chronic Kidney Disease (CKD) is a term used to describe a gradual reduction in renal function over a span of few weeks to years (Cheng and Vijayan, 2013) and manifested by proteinuria (Jafar et al, 2003; Donadio et al, 2003; Atkins et al, 2005) hematuria (Gutierrez et al, 2007; Cox et al, 2012) of glomerular or interstitial origin in the setting of a normal or higher than normal Glomerular Filtration Rate (GFR) eg., diabetic nephropathy in early stages manifested by proteinuria or IgA nephropathy (Flog and Eithner, 2011) with hematuria (Levey et al, 2007).

Uremia is “urea in the blood”, the primary components of urine. It is due to the accumulation of end products of amino acid and protein metabolism like urea and creatinine which is to be excreted from the body through urine (Aronov et al, 2011; Eloot et al, 2011 and Meyer and Hostetter, 2012). The terminal clinical manifestation of kidney failure is called as uremic syndrome (Bishop et al, 2010). It refers to the signs and symptoms due to inadequate regulatory, excretory and endocrine functions of the kidneys (Burtis et al, 2007). Uremia is the pathological manifestation of severe azotemia (Bishop et al, 2010). It is associated with the imbalances of fluid, hormone and electrolyte with metabolic abnormalities which occurs in parallel with worsening of renal function (Chikota, Gunderman and Omen, 2006). Azotemia also refers to the higher urea concentration in the blood which can be measured chemically and does not produce symptoms (Parikh and Coca, 2010).

Uremia is the condition which encompasses all the signs and symptoms of advanced kidney disease (Vanholder et al, 2003). Uremia signs and symptoms
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occur due to accumulation of organic waste products that are not excreted by the kidneys (Meyer and Hostetter, 2007). The uremic symptoms occur when the Glomerular Filtration Rate (GFR) goes below fifty percent of normal kidney function i.e., with less than 60 ml per minute per 1.73 m² of body surface area (National Kidney Foundation Kidney Disease Outcomes Quality Initiative NKF KDOQI, 2000; Mogensen 1999 and Vaidya et al, 2011). The most important organic solute that is identified in the blood of subjects with kidney failure is urea (Duranton et al, 2010; Meyer and Hostetter, 2007 and Rhee et al, 2010).

The pathogenesis of protein wasting in chronic kidney disease is multifactorial (Jadeja and Kher, 2012; Alice et al, 2011 and Carrero et al, 2011). International Society of Renal Nutrition and Metabolism (ISRNM) expert panel defined protein energy wasting during kidney failure as “state of decreased body stores of protein and energy fuels (body protein and fat masses)” (Fouque et al, 2008). Nowadays in the clinical setting of kidney disease the term ‘cachexia’ has been proposed to indicate protein-energy wasting (Kalander-Zadeh, 2005). The ISRNM panel members have suggested the use of cachexia for a severe form of protein energy wasting (Fouque et al, 2008).

Anorexia is the reason for decreased energy and protein intake (Burrowes et al, 2005; Carrero et al, 2007; Kalantar-Zadeh, 2004; Lopes et al, 2007). Anorexia may be facilitated by many factors such as gastric mediators like peptide YY (Perez-Fontan et al, 2008), ghrelin (Muscaritoli et al, 2007) or obestatin (Carrero et al, 2011), adipokines like visfatin (Carrero et al, 2010) and leptin (Cheung et al, 2005), cytokines such as interleukin[IL]-6 and IL-1β (Carrero et al, 2007 and Kalantar-Zadeh, 2004).

Low appetite (Carrero et al, 2008 and Evans et al, 2008) or poor absorption of nutrients plays a significant role in etiology of protein energy malnutrition. Anorexia in uremic subjects reduces the oral intake of energy and proteins thereby lead to protein energy malnutrition and cachexia (Bossola et al, 2006).
Metabolic acidosis increases protein degradation and amino acid oxidation in uremic subjects. Several studies had shown that correction of acidosis decreases protein breakdown (Graham et al., 1997). Metabolic acidosis is the principle factor for starvation mechanism, inducing release of branched-chain amino acids from muscle during ketosis. It also causes insulin resistance leading to loss of muscle mass (Bailey et al., 2005).

Dietary protein intake has greater impact on progression on renal disease (Metges and Barth, 2000). The habitual consumption of dietary protein more than the recommended levels aggravates the kidney disease through increased hyper filtration and glomerular pressure (Metges and Barth, 2000). The relationship between the renal function and dietary protein has been extensively studies. In 1923, Addis and Drury were among the first to observe a relationship between level of dietary protein and urea excretion rate and they demonstrated that renal blood flow was the basis for Glomerular Filtration Rate (GFR) mediated changes rates in response to increased protein intake (Tuttle et al., 2002).

Dietary protein has numerous fates. Initially, the dietary protein breakdown to yield amino acids that is required for the protein synthesis. Secondly, the digestion of protein will result in nitrogen containing waste products which has to be excreted from the body and if not excreted it gets accumulated to cause uremia. Urea – the predominant nitrogen-containing metabolite derived from dietary protein serves as the indicator for the degree of accumulation of uremia related toxins (Masud et al., 2002). Diet rich in high protein results in hyperuricemia, which not only increases the risk of gout, but has also been associated in the development of hypertension, metabolic syndrome and severe endothelial dysfunction with vascular disease (Choi et al., 2004; Cirillo et al., 2006; Feig et al., 2006 and Khosla et al., 2005).

Adam-Perrot et al (2006) stated that relation between dietary protein intake and renal function and Crowe (2005) reported that there is an increased risk of renal dysfunction when the diet is extremely high in protein content. This mechanism is attributed to trigger the renal hyper filtration to damage the renal

Theoretically uremia can be treated by reducing the production of solute. High dietary protein intake increases the production of uremic solutes such as indoles, guanidines and phenols. Physicians has observed that protein restriction in patients suffering from kidney disease, relieved the symptoms of uremia (Kopple et al, 1999).

Studies on uremic rat models suggest the predisposing factor for protein energy malnutrition from the existing literature was increased protein catabolism and decreased protein synthesis (Bammen et al, 2003). Absorption of dietary protein should be studied in the uremic rat models to determine the essential amino acid absorption for protein synthesis.

Besides the intellectual satisfaction of learning how CKD stimulates the loss of body weight and influences the “intracellular milieu”, it was believed that understanding mechanisms which underlie metabolic abnormalities in protein and amino acids is the first step towards devising strategies to block or ameliorate such defects (Kopple et al, 2013).

The World Health Organization recommends a “safe level intake” of 0.75 g/kg/day dietary protein to maintain nitrogen balance in healthy subjects under normal circumstances (WHO, 1985). But the available data was not convincing for the evidence related to the requirement of dietary protein for the different age groups. Campbell et al., (2008) showed that the mean protein requirement was not different between younger (21–46 years) and older (63–81 years) healthy adults.
Tovar-Palacio (2011) studied the effect of different amount and types of protein intake in obese Zucker rats (normal renal function) and reported that long term consumption of high protein diet are detrimental to the kidneys.


The term “availability” and “digestibility” are the key factors in determining the dietary protein quality. Digestibility refers to the combined effects of digestion and absorption but it does not give any information on extent of utilization of the absorbed nutrients (Mcnab, 1976).

Availability is defined as a nutrient entering the living tissue where it can potentially useful for metabolic functions of protein maintenance and protein synthesis. Bioavailability of dietary amino acids plays a predominant role in the protein synthesis which ultimately helps in treatment of protein energy malnutrition. Bioavailability hence refers to the amount of each amino acid which can be potentially be utilized for body protein synthesis and other anabolic processes, following the successive step of digestion, absorption and metabolism.

Carpenter (1973) reported that digestibility values of nitrogen and amino acids may overestimate the values of availability, in the food materials which are subjected to excessive heating during processing. Williams et al (1987) tested two different sources of protein derived from either animal or vegetable origin such as casein or soya in stable CKD rat and studied that after three months of dietary regime, glomeruloscelrosis and tubular dilation were found to be significantly greater in the casein versus soya fed group. Proteinuria was greatest with the casein group compared to soya fed group. They reported that due to different digestibility of protein, it is possible that vegetable proteins were less absorbed by about 10 per cent than the animal proteins.
Food and Drug Administration (FDA, 2015) defines dietary supplement is a product intended for ingestion that contains “dietary ingredients” intended to add further nutritional value to (supplement) the diet. A “dietary ingredient” may be one or any combination of the following substances: a vitamin, a mineral, an herb or other botanical, an amino acid, a concentrate or metabolite or constituent or extract, a dietary substance for use by people to supplement the diet by increasing the total dietary intake.

Many studies demonstrated that proper dietary protein management helps in the treatment of kidney disease. Dietary management reduces the uremic toxicity along with the reduction of signs and symptoms of uremia, minimizes the accumulation of waste metabolite products, prevents secondary hyperparathyroidism, protects against proteinuria and hypertension which ultimately slows the decline of residual renal function and progression of renal disease in the subjects with renal insufficiency (Barsotii et al, 1996 and Barssttis et al, 1983).

Good dietary management provides a temporary stabilization or sometimes exhibits improvement in renal function for months or years and can delay the progression of kidney disease and the time of initiating the replacement therapy like dialysis and transplant, subsequently reducing the overall cost of treatment (Bergstrom, 1994). Despite many studies focused on the dietary management of chronic kidney disease, studies on protein intake are very minimal and optimal protein intake has not been estimated so far (Bergstrom et al, 1988).

Research is needed to determine which protein can be restricted and what type of protein can be given to achieve the maximal effect in controlling the progression of renal disease without compromising the growth of the individual (Arije et al, 2002).

Since ancient times, egg has been used as a food for human consumption. It has nature’s perfect protein with other high quality nutrients. Egg can be used as whole, as yolk or as white fractions. Egg possesses good
functional properties and good nutritional value with good sensory characteristics that all are of big importance for food application (Kato et al., 1993). Eggs are said to be ‘polyfunctional’ as they contribute with more than one functional property at the same time (Pomeranz, 1991). The protein content of egg white is around eleven percent and contains eighty five per cent water. Approximately 40 different types of proteins are found in the egg white. There are four layers in the egg white with similar protein composition, except the viscous layers consist of higher ovomucin (Coultate, 2009).

Small intestinal assimilation of dietary protein, providing essential and nonessential amino acids for protein synthesis, plays a pivotal role in the maintenance of total body nitrogen balance. Information on the efficiency of protein assimilation in uremia is limited, most probably due to the lack of easy and reliable measuring technique (Mosenthin et al, 1994).

Food and Agricultural Organization / World Health Organization (FAO/WHO, 2011) expert consultation suggests that it is difficult and expensive to determine true ileal amino acid digestibility directly in humans. Hence there is a need for animal models to determine the amino acid digestibility. It was agreed that the rat, a nocturnal, meal eating omnivore possess similar anatomical structure of digestive tract (mouth to ileum) and have similar digestive physiology to adult humans.

Egg white protein is considered as high biological value protein with all indispensible amino acids necessary for protein synthesis. Hence the study “In-vivo evaluation of protein supplement in the management of uremia” was framed and carried out with the following primary and secondary objectives respectively:

**Primary objectives**

- To evaluate dietary (egg white powder) protein digestibility in uremic rat model.
Secondary Objectives

To

- Formulate cookies incorporated with egg white powder.
- Evaluate (*in-vivo*) protein quality of the formulated cookies and other experimental feeds
- Evaluate the effect of formulated cookies with respect to changes in renal biochemical parameters

It is hoped that this study will bring to light the dietary protein absorption in the uremic condition and paves the way to determine on what type of protein to be prescribed to the subjects suffering from kidney disease and thereby help to understand how far the indispensible amino acids are being absorbed from the diet.

Need for the study

- Understanding the absorption of indispensible amino acids (from dietary origin) during the initial stage is very important to prevent the prolongation of kidney disease
- This paves the way to emphasize the quality and quantity of protein to be advised during such conditions.
- This may in turn help to prevent the uremia related metabolic changes and reduce the risk of morbidity and mortality.

Hypothesis

This study is postulated with the null hypothesis that

- The formulated cookies incorporated with Egg White Powder does not show any effect on Indispensible Amino Acid absorption at terminal ileum in the uremic rat model
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- The formulated cookies does not have any significant effect on serum albumin levels in the uremic rat model.

- The formulated cookies does not have any significant effect on serum electrolytes levels in the uremic rat model.

- The formulated cookies does not have significant change in the overall health status.