"The processes of scientific discovery is, in effect, a continual flight from wonder"

-ALBERT EINSTEIN

German physicist
Chapter I

INTRODUCTION

Lactation is the production of milk. The mammary glands contain milk glands. In the glands, special epithelial cells line small sacs called alveoli. These cells secrete milk. The alveoli are surrounded by a layer of tissue containing smooth muscle fibers. When the muscle contracts it causes milk to be released. Milk enters a series of ducts (tubes), and each duct has an expanded space called a sinus which stores milk. The ducts eventually pass to separate openings in the nipple (Taylor D.J., 1984).

The breasts increase in size during pregnancy due to the development of the milk glands, controlled by progesterone, and ducts, controlled by estrogen. Human placental lactogen, another hormone, is also involved. However, for milk to be produced, the hormone prolactin must be present. This is secreted by the anterior pituitary gland. Throughout pregnancy the presence of estrogen and progesterone inhibits the secretion of prolactin and therefore the formation of milk. At birth, when the estrogen and progesterone levels fall due to loss of the placenta, prolactin is no longer inhibited and it stimulates the alveoli to secrete milk. (Falconer, I.R. 1980).

The lactation provides all the essential components of nutrition required by the newborn, namely high quality proteins, fats, lactose, essential minerals, electrolytes and vitamins. It also contains high concentrations of secretary IgA antibodies, which confer local gastro-intestinal immunity to the newborn child. Pregnancy induced hypertension (PIH) affects the functions of a numbers of organ systems which subsequently affect both lactogenesis and the composition of colostrum. Also maternal nutritional status has been found to influence the composition of milk. Malnourished mothers often produce milk which is sub-optimal in quantity and quality with deficiencies in proteins, calcium and fat along with certain immunological substances like IgA (Thylefors, 1985).

Although breast development begins around puberty, development of mammary function is only completed in pregnancy. During the first half of pregnancy the mammary ducts proliferate and group together to form large lobules. During the second half of pregnancy, secretory activity increases and the alveoli become distended by accumulating colostrum. After 16 weeks of pregnancy, lactation occurs even if the pregnancy does not progress.
The ability of the mammary gland to secrete milk during later pregnancy is called lactogenesis, stage 1. During this time, breast size increases and fat droplets accumulate in the secretory cells. The onset of copious milk secretions after birth is lactogenesis, stage 2, and usually occurs from day two or three to eight days postpartum. During this time, the milk goes through a maturation process to match the infant's needs. Without the hormone prolactin, lactation would not occur.

During pregnancy prolactin helps to increase breast mass but does not cause lactation because it is inhibited by the hormone progesterone, which is made by the placenta. The inhibiting influence of progesterone is so strong that lactation is delayed if any of the placenta is retained after birth. Prolactin levels rise and fall in direct proportion to the frequency, intensity, and duration of nipple stimulation from the infant's suckling. During the first week after birth, prolactin levels in breastfeeding women fall about 50 percent. If a mother does not breastfeed, prolactin levels usually reach the levels of the nonpregnant state by seven days postpartum. After milk "comes in" or rapidly increases in volume, lactation is no longer driven by the hormone prolactin. It shifts control to a milk removal driven process, i.e., sucking stimulus. Thus, the initiation of lactation is not driven by breastfeeding, but breastfeeding is necessary for the continuation of lactation.

The breast is not a passive container of milk. It is an organ that actively produces milk due to the stimulus of the infant's sucking; the removal of milk from the breasts causes continued milk production. It is a supply and demand response that regulates the production of milk to match the intake of the infant. The composition of breast milk changes to meet the specific needs of the growing infant. In response to suckling, the hormone oxytocin causes the milk ejection reflex or "let-down" reflex to occur. Milk ejection is the forceful expulsion of milk from the alveoli openings. Oxytocin secretion is also nature's way of causing a woman's uterus to contract after birth to control postpartum bleeding and assist in uterine involution. These contractions can continue for up to 20 minutes after feeding and may be painful during the first few days. The benefit of this, however, is that uterine discharge diminishes faster and the uterine involution occurs more quickly.

Secretion of the mammary gland at the end of pregnancy and at the beginning of lactation is known as colostrums. Unlike milk, it is yellow in color and is secreted from the breast only
for few days after the birth of child. Colostrums contain little fat but numerous cell fragments, free-fat droplets, lymphocytes, monocytes, histocytes, desquamated epithelial cells and colostrum corpuscles. It is rich in the protein globulin, but low in fat. It is believed to be a means of passing antibodies particularly IgA from mother to baby. (Mace, Hadely, 1984).

Colostrum is thick and creamy yellow as compared with mature milk, which is thin and bluish-white. Compared with mature milk, colostrum is richer in protein and minerals and lower in carbohydrates, fat, and some vitamins. The high concentration of total protein and minerals in colostrum gradually changes to meet the infant's needs over the first two to three weeks until lactation is established. The key component in colostrum and breast milk is immunoglobulins or antibodies that serve to protect the infant against infections or viruses. Breast milk also facilitates the development of the infant's own immune system to mature faster. As a result, breast-fed babies have fewer ear infections, diarrhea, rashes, allergies, and other medical problems than bottle-fed babies.

Human milk is rich in proteins, lipids, carbohydrates, vitamins, minerals, hormones, enzymes, growth factors, and many types of protective agents. It contains about 10 percent solids for energy and growth and the rest is water, which is essential to maintain hydration. This is also why a breastfed baby does not need additional water. Infants can digest breast milk much more rapidly than formula and, therefore, do not get constipated. On average, it takes about 30 minutes longer to digest formula as opposed to breast milk. Breastfed babies have better cheekbone development and better jaw alignment.

Transitional milk is secreted between about four days and ten days postpartum. It is intermediate in composition in between colostrum and mature milk. The volume increases during this time.

Mature milk is produced from approximately ten days after delivery up until the termination of the breastfeeding. Mature milk contains on average:

Besides the benefits of the contracting uterus, the process of producing milk burns calories, which helps the mother to lose excess weight gained during pregnancy. After all, that is why pregnant women put on extra fat during pregnancy energy storage for milk production. Breastfeeding is also related to a lower risk of breast cancer and ovarian cancer. For every year of
life spent breastfeeding, a woman's risk of developing breast cancer drops by 4.3 percent and this is on top of the 7 percent reduction she enjoys for every baby to whom she gives birth.

Additionally, there is the convenience. Breast milk is always with the mother. Mothers do not have to store it. It is always at the right temperature. It is free. It does not require sterilization. In fact, it prevents diseases and has protective factors resulting in healthier babies and decreased healthcare costs. It saves money as there is no need to buy formula, bottles, and nipples.

Human milk is the finest source of nutrition for human infants. During the first week postnatally, human milk is filled with immunoglobulins (IgG, IgA, and IgM), interferons, and other antibacterial substances which results in colonization of the GI tract of the newborn with benign bacterial flora. The presence of numerous growth factors (IgF-1), cytokines and gastric hormones enhance development of protective barriers in the GI tract and stimulate elimination of meconium present during gestation. Other key benefits to the infant include perfect nutrition, enhanced neurocognitive development, stronger immune function, and at least a three fold reduction in infectious disease such as upper respiratory infections, otitis media, and necrotizing enterocolitis.

Milk is produced in the alveolus. The alveolus is made up of gland cells around a central duct. The milk is produced by the gland cells. Surrounding the gland cells are the myoepithelial cells which contract to cause milk ejection into the milk duct. The milk then travels down the lactiferous ducts.

Figure 1
Lateral view with anatomical overlay A cross-section view of the alveolus.
Milk is stored largely in the alveoli with little storage in the ducts between breastfeeding. Mothers continue to make milk between feedings and they make more milk during feedings (Hale, 2007). When an infant breastfeeds, the infant draws the nipple and the areola into their mouth. The mother's nipple elongates to about twice its normal length. The nipple height is compressed between the tongue and the palate. Milk is ejected about 0.03 seconds after maximum nipple elongation (Smith, 1988).

Suckling infants compress the areola with their gums which stimulates oxytocin release. The oxytocin release causes an increase in the diameter of the milk ducts and movement of milk in the ducts toward the nipple. Mothers may feel a sensation of pins and needles, pressure, or pain in the breast with milk ejection. This sensation may disappear as lactation continues over a several month period of time. Using ultrasound examination of the breast in women who had been breastfeeding for at least 1 month, Ramsay and others found that initial milk ejection as manifested by an increase in diameter of the milk duct and movement of the milk fat globules, occurred at an average of 50 seconds after suckling began. The number of milk ejections that a woman had during a breastfeeding varied from 1 milk ejection in 26% of the women to 2-9 milk ejections in the other women. The mean number of milk ejections was 2.5 ejections/breastfeed. Between milk ejections, the diameter of the milk duct returned to the pre-ejection diameter. This suggests that milk storage does not occur in the larger milk ducts, but in the smaller ductules. Mothers who noticed breast sensations with the first milk ejection reported no breast sensations with subsequent milk ejections (Ramsay, 2004). In order to produce milk, hormones are needed. The two main hormones are prolactin and oxytocin.
Prolactin is produced by the adenohypophysis (anterior pituitary) and released into the circulation. The regulation of prolactin levels in the plasma is controlled by the dopaminergic system. Prolactin acts on the human breast to produce milk. This occurs by binding to mammary epithelial cell receptors, which stimulates synthesis of mRNA of milk proteins (Lawrence, 2011). It takes several minutes of the infant sucking at the breast to cause prolactin secretion. Prolactin is also important in inhibiting ovulation.

Oxytocin is produced by the neurohypophysis (posterior pituitary). Suckling at the breast stimulates the neurohypophysis to produce and release oxytocin in an intermittent manner. Oxytocin acts on the breast to produce milk ejection or "milk let down." Oxytocin also causes uterine contractions. Opiates and endorphins released during stress can block the release of oxytocin (Lawrence, 2011). Newton showed that women who received a saline injection and were distracted during breastfeeding produced less milk than women who were not distracted or women who received an injection of Pitocin (synthetic oxytocin) prior to distraction and breastfeeding (Newton, 1948, Newton, 1992). Lack of release of oxytocin inhibits the "milk let down" and the milk cannot be removed from the breast (Neville, 2001).

Other hormones necessary for the production of breast milk include: insulin, cortisol, thyroid hormone, parathyroid hormone, parathyroid hormone-related protein, and human growth hormone.

A recently described hormone, Fil (feedback inhibitor of lactation), seems to play an important role in regulation of milk supply. Fil acts locally within each breast. Fil is secreted into breast milk. When the breast is not emptied, Fil remains in contact with the alveolar cells. Fil appears to act on an apical receptor on the alveolar cell. This inhibits secretion of milk constituents. The complete mechanism is not yet understood, however this appears to be the mechanism of decreased milk production due to not emptying the breast (Wilde, 1998, Peaker, 1998).

Both male and female infants may have palpable breast tissue at birth. This is due to the stimulation of the breast by maternal hormones during pregnancy. By two to three months of age the breast tissue regresses. Madlon-Kay found that 38 of 640 infants had galactorrhea ("witch's milk") secretion at some time during the first two months of life. The galactorrhea was most likely to be present in the first two weeks of life (Madlon-Kay, 1986).
A woman's breasts grow during puberty in response to hormones. These hormones include: prolactin, estrogen, progesterone, cortisol, insulin, thyroid hormones and growth hormone. During puberty the release of these hormones causes proliferation of the lactiferous ducts and the development of breast tissues.

During pregnancy breasts increase in size due to an increase in lobules and alveoli. The release of estrogen and progesterone from the placenta and prolactin from the adenohypophysis causes the breast development. Women's breasts are prepared to produce milk as early as the sixteenth to the twentieth week of gestation. Breast milk production is inhibited during pregnancy by the effect of progesterone on prolactin.

Milk production is initiated in the breasts in the post-partum period due to prolactin production and decreased estrogen and progesterone after delivery of the placenta. The onset of lactogenesis (the production of breast milk) has been shown to be delayed by stressful events around delivery. Women who underwent an urgent Cesarean section or had a long duration of labor before vaginal deliveries were more likely to have a delayed onset of breast fullness in the first days after delivery (Dewey, 2001).

Mothers who have breastfed previously and have adopted a child have been successful in producing milk based on stimulation of their breast alone by the infant suckling at their breast. This is a slow process and some mothers may never produce enough milk to totally feed their child. This is termed relactation.

Women who have never been pregnant and whose breasts have not undergone the hormonal preparation of pregnancy may produce a small amount of milk based only on infant suckling at the breast. However, these women usually produce less milk than women whose breasts have been primed for breastfeeding by a prior pregnancy.

Human milk contains fat, lactose (milk sugar) and the proteins lactalbumin and casein which are all easily digestible. The milk is made from nutrients circulating in the blood, such as lactose from glucose, proteins from amino acids, and fats from fatty acids and glycerol. Milk alone is adequate to produce weight gains in the baby of 25 – 30g per day. In between breast feeds Prolactin stimulates milk production for the next feed (Short, R.V. 1976).

Breast feeding not only has health advantages, but also helps to create a close bond between mother and baby. Breast milk contains all the vital nutrients the baby needs to develop.
and grow into a healthy one. The vitamins, minerals, protein and fat content of breast milk are ideally balanced in a form that the baby can easily digest. By breast feeding the antibodies are directly transmitted to baby. It helps strengthen the baby’s immune system and promotes resistance to infectious diseases.

Breast feeding helps uterus shrink back its normal size. There is evidence that longer-term breast feeding may help to reduce postnatal weight. Women who have breast fed may have a reduced risk of premenopausal breast cancer, ovarian cancer and hip fractures due to osteoporosis.

Recent evidence from the world health organization has shown that breast feeding is more than 98% effective as a birth control method if the mother is a full time breast feeder and feeding on demand day and night.

The closeness and comfort of breast feeding is enjoyable for both mother and baby. The baby feel secure and contented and mothers can feed relaxed, peaceful and calm during and after breast feeding (Arlington, V.A, USA, Vital, 1991).

Mastitis is the most economically significant disease of dairy animals. This condition is widespread in dairy herds and is associated with a significant reduction in milk yield, increased costs of production and deteriorated milk quality. These costs are borne directly by milk producers and indirectly by the consumers of dairy products. The disease also results in partial or complete damage to udder tissues and decreases the productive life span of the animal. Mastitis is caused by many bacteria, which include the coliform group (specifically *Escherichia coli*, *Enterobacter*, *Klebsiella* species), *Streptococci*, *Staphylococci*, *Corynebacteria*, *Pasteurella*, *Mycoplasma*, *Leptospira*, *Yersinia*, *Mycobacteria*, *Pseudomonas*, *Serratia* and other organisms like fungi, yeasts and virus. (Kotowshi, 1988 and Gonzalez et al., 1980).

Gram-negative bacteria may be isolated from virtually any surface area of the cow or her surrounding and cause a host of diseases other than mastitis. Coliform bacteria are among the etiological agents commonly responsible for infectious respiratory and urogenital diseases in dairy cows. However, the spread of Gram-negative bacteria from other regions of the body to the mammary gland via the vascular or lymphatic systems appears minimal. Intramammary infections caused by Gram-negative bacteria typically result from the bacteria traversing the teat canal and multiplying in the gland and evade host defenses. Although the mammary gland is not
considered a natural habitat for coliform bacteria, many strains are capable of surviving and multiplying in the mammary gland. (Joe et al., 2003).

Coliform mastitis prevails more during the transitional phases of the non-lactating (dry) period than during lactation. The ability to infect the non-lactating gland is directly related to the ability of bacteria to acquire iron from the mammary secretion. The primary host defense against coliform mastitis during is the elimination of bacteria by neutrophils migrating into the gland in response to inflammation. Damage to the host is mediated by the release of endotoxin.

**Scope of the present investigation**

The significance of the proposed work based on the beneficial of breast milk through various biochemicals, Microbiological and molecular methods in lactating women. Hence this study was aimed to recruiting women who present with risk factors of lactation provided a very accurate prediction in mothers. This demonstrates the importance of counseling mothers with common known risk factors for Pregnancy induced hypertension (PIH), glucose intolerance and anemia in strategies that can be undertaken to prevent this significant. Awareness about the changes that occur in the composition of colostrum during complicated pregnancies can be an important and useful tool for preventive and protective pediatrics. The need for public health enlightenment of lactating mothers regarding hygiene, and the provision of oral vitamin A supplement is discussed.

**AIM AND OBJECTIVES**

- The aim of this study is to quantify the biochemical parameters in lactating women blood samples compare to non lactating women. Hence the biochemical parameters Serum Total protein, Serum Albumin, Serum Vitamin – A, Serum Vitamin – C, Serum Vitamin – B<sub>12</sub>, Serum Iron, Serum Calcium, Serum IgA, Serum Prolactin are estimated.
- To study the three groups of lactating women having the following complications categorized as: Group a) women with pregnancy induced hypertension; Group b) women with glucose intolerance; and Group c) women with anemia.
- The following parameters were analyzed in each group of samples in colostrum: Total proteins, Total lipids, Triglycerides, cholesterol, glucose, Calcium, inorganic phosphorus, IgA.
• To study the vitamin A content of colostrum, matured milk and transitional milk exhibited the same pattern as protein, calcium and sugar content.

• To study the microbiological and biochemical analyses of 40 breast nipple swab samples and 40 manually expressed breast milk samples of lactating mothers aged 15 to 35 years and 40 cow milk samples are analyzed.

• To study the incidence of bacterial species in swab samples, breast milk and cow milk samples are further confirmed by PCR 16s rna sequencing analysis.

• To identify the virulent *Ecoli and Enterobacter cloacea* from Bovine coliform mastitis in cow and to study the pathogenic property of *Ecoli* by PCR and 16s rna sequencing for virulent genes.