2.0 LITERATURE REVIEW

2.1 Thyroid gland

The thyroid gland is located in anterior part of the lower neck, below the larynx (voice box). It is the first glandular tissue to appear during embryological development in mammals. It arises from two regions of the endodermal pharynx which later develops into a bi-lobed structure (Park & Chatterjee, 2005). In adults, the normal thyroid gland is presented by two lobes joined across the trachea by the isthmus. Each lobe pre-lateral surface is covered by the infrahyoid muscles (Summers, 1950). The thyroid gland consists of three major types of epithelial cells. These include follicular cells, which line the follicles and secrete thyroxine and triiodothyronine, calcitonin-producing parafollicular cells (C cells) which secrete calcitonin, and the solid cell nest (SCN), which are remnants of the ultimobranchial body. The mean weight of the thyroid gland for an adult is about 20 gram (Hoyes & Kershaw, 1985; Kalina et al., 1971; Takasu et al., 1992).

2.2 Thyroid hormones

Thyroid hormones, triiodothyronine (T₃) and thyroxine (T₄) are produced from the thyroid gland and are involved in the coordination of a multitude of physiological responses and developmental processes in all vertebrates (Yen, 2001). In general, thyroid hormone synthesis involves the following steps: 1) thyroid stimulating hormone (TSH) binds to thyroid stimulation hormone receptor (TSH-R) and stimulates the iodide (I⁻) transport from the blood plasma into the thyroid gland by NIS; (2) I⁻ uptake by the follicular cells: (3) oxidation of I⁻ and iodination of tyrosine; (4) coupling of two
iodotyrosine to form hormonally active iodothyronines, 3, 5, 3’-triiodothyronine (T3) and 3, 5, 3’, 5’-tetraiodothyronine or T4. The steps involved in thyroid hormone synthesis are shown in Figure 2.1. T3 is structurally similar to T4 and has three atoms of iodine instead of four. Most T3 are produced by phenolic ring 5’- monodeiodination of T4 in extrathyroidal tissues (Granner, 1999). About 90% of the thyroid hormone released from the thyroid gland is in the form of T4. However, most of the secreted T4 are converted into T3 which is 10 times more potent in its biological activity (Sherwood & Cengage Learning (Firm), 2010). These two hormones play an important role in the regulation of skeletal development and growth. Deficiencies of these hormones result in hypothyroidism. Hypothyroidism during childhood causes developmental delay, growth retardation, delayed bone age and epiphyseal dysgenesis (O'Shea et al., 2003). In contrast, excessive amount of thyroid hormones leads to hyperthyroidism, which causes an increase in the metabolic rate, weight loss despite good appetite, excessive sweating, irritability, anxiety, tremulousness, palpitations, goitre and proximal muscle weakness (Sapini Y, 2010; Whitley, 1999).

2.3 Hypothyroidism

Hypothyroidism is a condition in which the thyroid gland does not produce enough functionally active thyroid hormones (Roberts & Ladenson, 2004). It is a relatively common disorder and arising more often in women than men (Eugene et al., 2005). Hypothyroidism can be divided into primary, secondary and tertiary subcategories. Primary hypothyroidism is characterised by a decreased production of thyroid hormones as a consequence of thyroid diseases. Common causes of primary hypothyroidism include functional problems within the thyroid gland, infiltrative
Figure 2.1 Schematic diagram of a follicular cell, illustrating the steps involved in thyroid hormone synthesis (Park & Chatterjee, 2005).
disease of the thyroid, silent or subacute thyroiditis, chronic autoimmune thyroiditis, radioiodine therapy, and postoperative hypothyroidism. External radiation to the neck of children or defects in any steps of thyroid hormone synthesis might also result in hypothyroidism. Secondary or tertiary hypothyroidism occurs when the problem lies within the pituitary or hypothalamus. The thyroid gland is intrinsically normal but it does not receive stimulation from the pituitary or brain to synthesise thyroid hormones. Secondary hypothyroidism can be acquired when pituitary gland does not produce and release adequate amount of TSH that is needed by the body. In tertiary hypothyroidism, problems within the hypothalamus cause decreased synthesis of thyroid releasing hormone (TRH) and subsequent decreased stimulation of the pituitary (Whitley, 1999). Hypothyroidism that is present at birth is known as CH whereas hypothyroidism in later life is called acquired or clinical hypothyroidism. Severe shortage of thyroid hormones after birth can lead to mental retardation and motor handicaps (de Vijlder, 2003). Meanwhile, hypothyroidism in later childhood and adults can result in a slowing of metabolic processes and is reversible with treatment. Severe untreated hypothyroidism can result in myxedema coma (Bonert & Friedman, 2007). Other common symptoms and signs of hypothyroidism are shown in Table 2.1.

2.4 Neonatal transient hypothyroidism

Neonatal transient hypothyroidism (NTH) or transient neonatal hypothyroidism (TNH) is a temporary form of hypothyroidism which can last from several days to several months after birth. The incident of NTH varies from 1 in 5845 in Iran to 1 in 700 in Belgium (Delange et al., 1978; Orduokhani et al., 2007). The causes of NTH remain unclear but factors including environmental, maternal, neonatal and genetic are
**Table 2.1** Clinical features of hypothyroidism (modified from Bonert & Friedman, 2007)

<table>
<thead>
<tr>
<th>Neonates and children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor feeding</td>
<td>Fatigue</td>
</tr>
<tr>
<td>Inactivity</td>
<td>Cold intolerance</td>
</tr>
<tr>
<td>Prolonged jaundice</td>
<td>Weakness</td>
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<tr>
<td>Tongue protrusion</td>
<td>Lethargy</td>
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<tr>
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<td>Weight gain</td>
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<tr>
<td>Constipation</td>
<td>Constipation</td>
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<tr>
<td>Learning difficulties</td>
<td>Myalgias</td>
</tr>
<tr>
<td>Mental retardation</td>
<td>Arthralgias</td>
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<tr>
<td>Short stature</td>
<td>Menstrual irregularities</td>
</tr>
<tr>
<td>Delayed bone age</td>
<td>Hair loss</td>
</tr>
<tr>
<td>Delayed puberty</td>
<td>Dry, coarse, cold skin</td>
</tr>
<tr>
<td></td>
<td>Coarse, thin hair</td>
</tr>
<tr>
<td></td>
<td>Hoarse voice</td>
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<tr>
<td></td>
<td>Brittle nails</td>
</tr>
<tr>
<td>Periorbital, peripheral edema</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Delayed reflexes</td>
</tr>
<tr>
<td></td>
<td>Slow reaction time</td>
</tr>
<tr>
<td></td>
<td>Orange skin hue</td>
</tr>
<tr>
<td></td>
<td>Bradycardia</td>
</tr>
<tr>
<td></td>
<td>Pleural, pericardial effusions</td>
</tr>
</tbody>
</table>
believed to be linked to NTH (Cheron et al., 1981; Delange, 1998; Lin et al., 1994; Niu et al., 2005).

2.5 Permanent congenital hypothyroidism

Permanent CH occurs in babies who are born without the ability to produce adequate amount of thyroid hormones. It is one of the world’s most common endocrine problems that affecting 1/3000 - 4000 newborn baby (Delange, 1979; Fisher et al., 1979). Permanent CH is mainly due to the primary hypothyroidism instead of central hypothyroidism (Van Vliet, 2003). About 80 to 90 % of CH cases are the result of dysembryogenesis of the thyroid gland. The remaining 10 - 20 % cases are due to thyroid dyshormonogenesis (Gruters, 1992). Symptoms and signs of CH in neonates include poor feeding, jaundice, hypothermia, bradycardia, an enlarged posterior fontanelle and umbilical hernia. However, most neonates with CH do not show significant symptoms and signs in the first few months after birth (Roberts & Ladenson, 2004). As a result, delay in diagnosis and treatment of CH cause these neonates to suffer from permanent neurologic abnormalities, growth failure and irreversible mental retardation (Gruters et al., 2004).

2.6 Biochemical screening and clinical diagnosis of CH

Most countries worldwide including Malaysia have newborn screening program to allow early diagnosis of CH. Screening of CH in newborns are carried out through the measurement of TSH levels in their cord blood or at 3 - 5 days of life and the results are further confirmed with serum thyroid function test (TFT), which measures the TSH, free T₄ (FT₄) or total T₄ levels. Normal serum TSH levels in the disease-free individuals
varies with age during the neonatal period and are as follows: less than 25.0 µIU/ml in cord blood; 0.4 - 10.0 µIU/ml after 5 days of life; 0.5 - 8.7 µIU/ml at a month of age; and 0.4 - 5.5 µIU/ml at 3 months of age and adult. The reference values of FT₄ are as follows: 20.0 - 68.0 pmol/L in cord blood and after 5 days of life; 23.0 - 54.0 pmol/L at a month of age; and 11.5 - 23.2 pmol/L at 3 months of age and adult (Musa et al., 2006). The reference value of total T₄ is between 60 - 140 nmol/L for both children and adults. CH is diagnosed when the neonate has elevated serum TSH level with low level of T₄ or abnormal total T₄ level. In Malaysia babies with cord blood TSH >25 µIU/ml are recalled to confirm the diagnosis of CH by repeating the TSH and FT4. If confirmed, they are given treatment with L-thyroxine as soon as possible and are regularly followed up for growth and developmental monitoring and dose adjustment of thyroxine as the children grow. However, neonatal screening using TSH alone can be insensitive in diagnosing CH cases which are due to hypothalamic-pituitary defect (Ross, 2001; Spencer et al., 1996). The status of the thyroid gland including size and location can be determined using thyroid ultrasonography or Technetium-99m (⁹⁹Tm) scintigraphy. Thyroid scintigraphy and ultrasonography are usually carried out before treatment is instituted. If this is not possible, most screening centres perform these imaging studies when the patients reached the age of 3 years, when it is safe to stop the T₄ replacement therapy temporarily before scanning (Rose et al., 2006).

### 2.7 Treatment of CH

The objective of the treatment is to maintain the thyroid hormones levels within the normal reference range required for metabolic processes throughout the body. Patients with CH can be treated with synthetic levothyroxine (L-T₄) or liothyronine (L-T₃) or a combination of the two synthetic hormones. Levothyroxine sodium is preferred
in thyroid hormone replacement therapy since liothyronine treatment results in fluctuations in thyroid hormone levels in the blood. The optimum dose of levothyroxine for hypothyroid patients is related to bodyweight and age. The starting replacement dose for CH patients is 25 - 50 µg per day or 10 - 15 mcg/kg of body weight per day for the first six months and is then readjusted according to clinical evaluation and serum FT4 and TSH levels in follow up examination. Patients with CH are advised to avoid soy products during the course of treatment as genistein in soy products can interfere with the absorption of the two synthetic hormones (Fruzza et al., 2012). Appropriate treatment of these patients will result in suppressed serum TSH levels and a normal FT4 or total T4 using the reference normal range for age (Bonert & Friedman, 2007; Toft, 1994).

2.8 Thyroid dyshormonogenesis

Defects in any of the steps during the thyroid hormone biosynthesis lead to dyshormonogenesis which typically manifests as CH and goitre (Gruters, 1992). Thyroid dyshormonogenesis is an uncommon cause of CH which only accounts for 10 - 15 % of total CH cases (de Vijlder et al., 1997). An autosomal recessive mode of inheritance has been noted in most reported cases with dyshormonogenesis. Majority of the patients with thyroid dyshormonogenesis have developed either a diffuse goitre or nodular gland at various phases of their lifetime in an attempt to compensate for the diminished capacity of the thyroid gland to produce thyroid hormones (Hulse, 1984). Most cases of goitrous CH with iodide organification defect are attributable to thyroid peroxidase (TPO) deficiency (Avbelj et al., 2007; Bakker et al., 2000; Deladoey et al., 2008). Thyroid dyshormonogenesis is also associated with defects in other genes that are involved in thyroid hormone synthesis such as dual oxidase 2 (DUOX2) (Varela et
al., 2006), *solute carrier family 5* or *sodium iodide symporter (SLC5A / NIS)* (Pohlenz & Refetoff, 1999), *thyroglobulin (Tg)* (Ieiri et al., 1991), and *thyroid stimulating hormone, beta (TSHB)* (Deladoey et al., 2003).

### 2.9 Iodide organification defects

Under normal condition, I\(^-\) will be oxidised and bind to the tyrosine residues in Tg, where this process is commonly referred to as iodide organification. However, in some cases, I\(^-\) in the thyroid gland cannot be oxidised and/or bound to the Tg protein and these defects can lead to thyroid problems like hypothyroidism. Iodide organification defects can be partial or total depending on the degree to which I\(^-\) can be organified. Partial organification defect (PIOD) is characterised by a discharge of more than 10 % of the radio-iodine taken up by the gland within one hour during perchlorate discharge test. In contrast, total iodide organification defect (TIOD) is more critical where this condition usually happens in patients with severe CH. These patients have shown a discharge of more than 90 % of radio-iodine after administration of sodium perchlorate within an hour. Iodide organification defects are usually due to either a shortage of any of these elements like hydrogen peroxide (H\(_2\)O\(_2\)), I\(^-\) and Tg during iodination process or disorders in *TPO* (de Vijlder, 2003; Perez-Cuvit et al., 1977).

### 2.10 Goitre

Goitre refers to the enlargement of the thyroid gland. Goitres are classified into three groups according to the functional status of the gland ie: euthyroid (simple goitre), hyperthyroid (toxic nodular goitre or Grave’s disease) or hypothyroid (iodine
deficiency, CH or Hashimoto’s thyroiditis). Goitre due to Grave’s disease characteristically has symmetrically enlarged gland with a smooth surface and often with a bruit. A diffuse, smooth goitre with negative anti-thyroid may be due to iodine deficiency, where the thyroid gland enlarges as it attempts to comply with the pituitary's demand. However, other thyroid problems like CH and other inflammatory conditions of the thyroid gland are also responsible for the formation of goitre. Meanwhile, a nodular thyroid gland with hypothyroidism and positive anti-thyroid antibodies is suggestive of Hashimoto’s thyroiditis (Bonert & Friedman, 2007). A large goitre often can cause swallowing and breathing problems. Even though the majority of goiters are benign, it is important to ensure that they are not due to underlying neoplasm. It must be recognised that thyroid carcinoma associated with dyshormonogenetic goitre have been reported (Alzahrani et al., 2006; Camargo et al., 2001; Chertok Shacham et al., 2012; Cooper et al., 1981; Medeiros-Neto et al., 1998).

2.11 Thyroid nodules

Thyroid nodules are common especially among women in area of iodine deficiency. Nuclear medicine thyroid scan on nodular function is used to differentiate “cold” and “hot” nodules. During the examination, the radioactive iodine acts like non-radioactive iodine and will be absorbed by the thyroid gland to be used in the production of thyroid hormones. Functional thyroid tissue will absorb the radioactive iodine and become radioactive. A nodule is hot or hyperfunctioning if it picks up more radioactive iodine than a normal tissue. In contrast, a cold nodule is less functional and usually will pick up less or none of the radioactive iodine. “Hot” nodules are almost always benign, whereas cold nodules may harbor cancer in a small proportion (< 10 %) of patients (Belfiore et al., 1992; Mackenzie & Mortimer, 2004; Sarkar & Becker,
Thyroid nodules are usually presented in benign form including follicular adenoma, colloid nodules, benign cysts or nodular thyroiditis. However, it is important to determine the status of the nodules as about 5% of them have shown to be malignant. Moreover, most thyroid cancers are of low grade malignancy and share pathologic features with benign nodules. Therefore, routine evaluation of the status of these nodules by fine needle aspiration biopsy (FNAB) and cytological examination are needed (Bonert & Friedman, 2007).

2.12 Follicular adenoma

Follicular adenoma is the most common benign tumor of the thyroid gland (Stevens et al., 2011). It is characterised by a firm, homogeneous, round or oval tumor that is surrounded by a thin fibrous capsule (Bisi et al., 1989; Silverberg & Vidone, 1966). It cannot be distinguished from follicular carcinoma without histological examination as both tumors share similar microscopic features. However, if compared to follicular carcinoma, follicular adenoma tends to be less cellular, has thinner capsule and lacks invasiveness (D’Avanzo et al., 2004). The ratio of follicular adenoma to follicular carcinoma in surgical specimen is approximately 5:1 (Rosai et al., 1991). Patients with follicular neoplasm (including follicular adenoma and follicular carcinoma) usually will be advised to undergo surgery to remove the thyroid nodule unless there is clear evidence that the nodule is hyperfunctioning (Mackenzie & Mortimer, 2004).
2.13 Thyroid peroxidase (TPO) gene

Thyroid peroxidase (TPO) gene is also known as tyroperoxidase (TPX), thyroid microsomal antigen (MSA) or thyroid dysshormonogenesis 2A (TDH2A), which is located on the short arm of chromosome 2, locus 2p25 (Endo et al., 1995). It consists of 17 exons and 16 introns which spans about 150 kbp in genomic DNA (Kimura et al., 1989; McLachlan & Rapoport, 1992). The expression of TPO is under the control of transcription factors such as thyroid transcription factor 1 (TTF-1), thyroid transcription factor 2 (TTF-2) and paired box gene 8 (PAX-8) (Damante & Di Lauro, 1994).

The full-length of the human TPO transcript (TPO1) is 3152 bp in size (GenBank accession number NM_000547.5), consists of 17 exons and codes for 933 amino acids (Kimura et al., 1987; Libert et al., 1987; Magnusson et al., 1987; Seto et al., 1987). Apart from TPO1, seven different TPO transcripts which are generated through alternative splicing had also been discovered. Differential splicing generates shorter transcripts: TPO2, TPO3, TPO4 and TPO5 lacking exons 10, 16, 14 and 8 respectively (Elisei et al., 1991; Ferrand et al., 2003; Zanelli et al., 1990). In addition, three other multi-spliced transcripts such as TPO2/3 (lacking exons 10 and 16), TPO2/4 (lacking exon 10 and 14), and TPO6 that lacks exons 10, 12 - 14 and 16 had also been described (Ferrand et al., 2003). A schematic diagram of these transcripts is shown in Figure 2.2. Among these eight types of TPO transcripts, only TPO1, TPO3 and TPO4 are expected to produce enzymatically active TPO protein and can therefore be expected to play important roles in thyroid hormone synthesis. TPO2, which is lacking in exon 10, leads to a production of TPO protein which does not have any enzymatic activity and is rapidly degraded after synthesis. TPO5 lacks exon 8 which has been suggested to code for a site that participates in catalytic mechanism. The protein
Figure 2.2 Human TPO mRNA transcripts. TPO1 is the full-length form of TPO mRNA. Variants lacking single exons are TPO2 (lacking exon 10); TPO3 (lacking exon 16); TPO4 (lacking exon 14) and TPO5 (lacking exon 8). Multi-spliced variants are TPO2/3 (exons 10 and 16 deleted); TPO2/4 (exons 10 and 14 deleted) and TPO6 (exons 10, 12, 13, 14 and 16 deleted).
translated from TPO5 is also unable to acquire a proper three dimensional configuration and reach the cell surface. In contrast, TPO4 which is lacking in exon 14 is able to fold correctly and reach the cell surface. However, this isoform has a shorter half life compared to TPO1 (Elisei et al., 1991; Ferrand et al., 2003; Zanelli et al., 1990).

2.14 Thyroid peroxidase enzyme

TPO is a 110 kDa membrane-bound, glycosylated, heme-containing protein. It catalyses the iodination of Tg and is involved in the coupling of some of the iodotyrosyl residues to generate active iodothyronines, T_{3} and T_{4}. TPO is mainly expressed in the thyroid gland and is localised on the apical membranes of the thyrocytes (Cetani et al., 1995; Chedrese, 2009; Gardas et al., 1997). A study has reported that TPO is also detected in orbital tissues (Lai et al., 2006). The three-dimensional (3-D) structure of this protein remains unknown although some low resolution crystals have been obtained (Gardas et al., 1997; Hendry et al., 1999). TPO shares 42 % sequence identity with myeloperoxidase (MPO) where it’s 3-D structure is known. Therefore, the high homology of these two proteins has allowed the prediction of the secondary structure and organisation of the TPO to be made. The structure of TPO that was deduced from alignments studies and structural homologies have shown that human TPO comprises three distinct modules: MPO-like, complement control protein-like and epidermal growth factor-like domains (Figure 2.3) (Banga et al., 1990; Bresson et al., 2005; Hobby et al., 2000; Libert et al., 1987).
Figure 2.3 Schematic diagram of the structure of human TPO. Human TPO is formed by myeloperoxidase (MPO)-like, a complement control protein (CCP)-like, and an epidermal growth factor (EGF)-like domains, from the N- to the C-terminal extremities (Bresson et al., 2005).

<table>
<thead>
<tr>
<th>N-terminal</th>
<th>MPO-like</th>
<th>CCP-like</th>
<th>EGF-like</th>
<th>C-terminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>142</td>
<td>736</td>
<td>796</td>
<td>848</td>
</tr>
</tbody>
</table>
2.15 TPO gene mutations and polymorphisms

The most prevalent cause of congenital dyshormonogenetic hypothyroidism is believed to be TPO deficiency (Mangklabruks et al., 1991). The TPO enzyme activity depends on two conditions: (1) proper folding and membrane insertion, (2) an intact catalytic site region (Ruf & Carayon, 2006). To date, at least 67 different types of mutation in the TPO gene have been described worldwide. Majority of the mutations are localised in exons 8, 9 and 10 that encode the catalytic heme-binding domain of the protein (Coleman & Tsongalis, 2009; Ris-Stalpers & Bikker, 2010). The reported mutations range included frameshift by single or multiple insertion or deletion of nucleotides, missense, alternative-splicing, nonsense mutations and total gene deletion. TPO defects are commonly inherited in an autosomal recessive mode (Park & Chatterjee, 2005) even though there was a reported case of a single TPO mutated allele in TIOD patients (Fugazzola et al., 2003). The complete list of TPO gene mutations is shown in Figure 2.4.

Single nucleotide polymorphism (SNP) is classically defined as a normal gene variants accounting for more than 1 % of alleles in a population. To date, 43 polymorphisms (31 non-synonymous coding and 12 synonymous coding polymorphisms) in the coding region of TPO gene have been documented. The summary of the published TPO gene polymorphisms is shown in Table 2.2.
Figure 2.4 A schematic diagram showing inactivating mutations reported in the coding region of the human TPO gene (Modified from Kang, 2010; Coleman & Tsongalis, 2009; Ris-Stalpers & Bikker, 2010).
Table 2.2 A summary of published TPO polymorphisms. Refer to the Emsembl genome annotation database for complete details, gene reference code: ENSG00000115705.

<table>
<thead>
<tr>
<th>Amino acid residue</th>
<th>Variation type</th>
<th>Codons</th>
<th>Residues</th>
</tr>
</thead>
<tbody>
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<td>Ala</td>
</tr>
<tr>
<td>4</td>
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2.16 Expression of TPO, Tg, TSH-R and NIS in thyroid nodules

Thyroid nodules are extremely common. Only about 5% of thyroid nodules have been shown to be malignant (Cooper et al., 2009). Therefore, it is important to determine the status of the nodules. Currently, it is achieved by FNAB as an initial test for diagnosing malignancy. However, FNAB does not give a conclusive result in some cases. As a result, many expression profiling studies have been carried out with the hopes of identifying new diagnostic markers.

Lazar et al. (1999) reported that later stages of thyroid cancer were associated with lower TPO mRNA expression. This finding was confirmed by other studies where a lower expression of TPO was commonly seen in thyroid carcinoma cases (Di Cristofaro et al., 2006; Finley et al., 2004; Hawthorn et al., 2004; Huang et al., 2001; Pauws et al., 2004; Prasad et al., 2008).

Tg is a large homodimeric glycoprotein that comprises 2768 amino acids (van de Graaf et al., 2001). It is responsible for the storage of the inactive form of thyroid hormones and iodine (Marino & McCluskey, 2000; Medeiros-Neto et al., 1993). The levels of Tg mRNA were significantly lower in adenoma and carcinoma tissues as compared to normal tissues in 1991 (Ohta et al., 1991). Contrary to that, other studies have shown that only thyroid carcinoma have lower expression of Tg (Karger et al., 2006; Ringel et al., 2001).

TSH-R was categorised under the superfamily of G-protein-coupled receptors that comprises 764 amino acids. It serves as a customised binding site for TSH. Binding of TSH to TSH-R stimulates thyroid epithelial cell proliferation. It also
regulates the expression of other thyroid-related genes such as Tg, TPO and NIS, which are required for the synthesis of thyroid hormones. Most of the well-differentiated carcinomas have different levels of TSH-R mRNA ranging from normal to markedly deceased. In contrast, majority of the undifferentiated anaplastic carcinomas are resulted in a loss of mRNA expression (Brabant et al., 1991; Bronnegard et al., 1994; Macchia, 2000; Sheils & Sweeney, 1999).

NIS is a transmembrane glycoprotein that consists of 643 amino acids. It plays an important role in regulating the level of I\(^{-}\) in the thyroid gland by actively transporting the I\(^{-}\) into thyroid epithelial cells (Levy et al., 1997). The expression of NIS is largely modulated by the expression and functional activity of TSH-R (Caillou et al., 1998). An increased expression of NIS was observed in papillary thyroid cancer specimens (Saito et al., 1998). Contrary to Seito results, other studies have demonstrated a downregulation of NIS expression in both benign and carcinomas tumors (Lazar et al., 1999; Ringel et al., 2001; Sodre et al., 2008). In contrast, increased expression of NIS has been demonstrated in patients with Grave’s disease and in autonomously functioning thyroid nodules (Castro et al., 1999; Jhiang et al., 1998; Joba et al., 1999; Sodre et al., 2008).