Investigation of testicular germ cell tumor on Cryptorchidism with Hypospadia and Ambigious Genitalia

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ABSTRACT

Objective: To identify germ cell malignancies and carcinoma in situ (CIS) in the gonads of high risk patients with cryptorchidism and hypospadias (DSD cases) in Semarang as well as the frequency of OCT3/4 presence in the case of germ cell malignancy.

Methods: Specimens were examined for routine histopathology H&E staining. Immunohistochemistry staining was performed using antibody OCT3/4 and SCF to evaluate germ cells maturity and its progress toward malignancies as well as its precursor lesion (CIS/GB).

Result: We collected 33 specimens, however only 27 samples which qualified with categorization of Germ Tumor. Stage of differentiation tissue was varied categorized ranging from ovary, sex cord (tubae or funiculars). There were 7/26 samples with malignancies and precursor lesion.

Conclusion: Genetic and environmental factors involve in the indirect role in the establishment of proper micro-environment for delayed maturation, transformation and progression of early germ cells. OCT3/4 immunostaining is a promising and useful tool in managing patients known to be at high risk for the development of invasive GCTs.

1. Introduction

Testicular germ cell tumours (TGCTs) are the most common malignancy in males between the age 15 and 45 years [1]. Recently, its incidence is becoming more prevalent [2]. Precursor lesion which is mainly composed of primordial germ cell/gonocyte, carcinoma in situ of testis (CIS) and gonadoblastoma of the dysgenetic gonad. The unique responsiveness of identity to DNA damaging agent is most plausible explained by embryonic characters which are retained by premalignant cells. Testicular cancer is the second most frequent cause of death in this age group are therefore refractory to its nature of treatment refractory [3].

There are several risk factors published for TGCT including previously diagnosed TGCT, undescended testis (UDT) and a family history of the disease. TGCT arises highest in the familial relative risks of any cancer syndrome with reported increased risks of 8–10-fold to brothers and 4–6-fold to fathers [4].

There is considerable variation in risk among several states with racial and ethnic groups are different. The highest incidence occurs among whites in the Nordic countries (1.5 per 100,000 men) and in contrast to the low incidence among populations of Asia and Africa (1-2 per 100,000 men). However, the Asian population alone there are differences in the incidence which happened quite low in East Asian populations (0.7-1.6 per 100,000) than in West Asia. (4.1 per 100,000)[5].
Ambiguous genitalia or Disorder of Sex Development (DSD) refers to a congenital disorder where there is an abnormality in the development of the sex chromosomes, gonadal or anatomical [6]. The disorder can occur at every stage of the determination and differentiation of sexual development may result in the occurrence of congenital genital malformations. For clinical purposes, DSD grouped into a) 46, XY DSD (formerly known as male pseudohermaphroditism or undervirilization); b 46, XX DSD (formerly known as female pseudohermaphroditism or masculinization, and c) Chromosomal DSD (including variants of Turner syndrome and Klinefelter syndrome [7]. TGCT (Testicular Germinal Cell Tumor) s are omnipotent, able to generate all differentiation lineages, both embryonic and extra-embryonic, as well as the germ cell lineage itself [8]. Recent data show that the incidence of testicular cancer in men who have had cryptorchidism is quite high ranging from 5-10% [9].

OCT3/4 has high sensitivity and specificity for CIS/gonadoblastoma, seminoma, and embryonal carcinoma, and is useful for the detection of CIS cells in semen, thus a promising tool for non-invasive screening. The development of CIS and gonadoblastoma is crucially dependent on the micro-environment created by Sertoli cells in the testis, and granulosa cells in the dysgenetic gonad. OCT3/4, also known as POUF5F1, a transcription factor that is essential for pluripotency of embryonic stem cells and primordial germ cells during normal development and is now recognized as a reliable marker for diagnostic CIS / DM [10].

OCT3/4 has mainly been linked to pluripotency, for which it is a well-known and established marker. Pluripotency refers to the capacity of a (embryonic) stem cell to generate all different tissues (endo-, ecto- and mesodermal differentiation) [11]. Stem Cell Factor (SCF) plays an important role in the process of migration, proliferation, survival, differentiation and apoptosis suppression Primordial Germ Cell / PGCs (Germ Cell Tumor cell origin) to the regulation of proliferation of spermatogonia in the adult testis [12].

Biopsy in cases of cryptorchidism were performed during childhood have a prognostic value because of the positive correlation between biopsy findings with abnormal spermatogenesis and finding lesions and carcinoma in situ germ cell malignancies. Therefore, the prognostic value of the fertility and the possibility of malignancy or precursor there of makes testicular biopsy procedure in cases cryptorchidism recommended and have proven that testicular biopsy procedure in childhood when doing orchidopexy does not cause adverse effects on the testes.

2. Materials and Methods

2.1 Research method

This study was conducted in January-October 2011. Samples were collected from the old and new patients who attended to Gender Adjustment Team, Center for Biomedical Research-dr. Kariadi General Hospital.

2.2 Study subject and research flow

Samples were selected by purposive sampling with the following inclusion criteria: (1) Abnormal phenotype with cryptorchidism with hypospadias or ambiguous genitalia patients with abnormal undervirilization phenotype and or gonadal dysgenesis (2) Hormonal and Cytogenetic test with gonadal dysgenesis and hypovirilization (chromosome of 46, XY and or mosaic with LH, FSH and testosterone at normal or with minimal increment level). (3) Willing to participate in the study. All of the study subjects were undergone thoroughly clinical examination performed by the urologist following the WHO classification [13].

2.3 Ethical clearance

Ethical clearance was granted by the Faculty of Medicine, Diponegoro University, Semarang-Indonesia (Ethical Clearance No.107/EC/ FK/RSDK/2010).

2.4 Histopathology evaluation

After the diagnosis and following orchidopexy/orchidectomy, samples were stained using Hematoxylin and eosin staining (H & E) for histopathological examination, supervised by anatomical pathologist.

2.5 Immunohistochemistry

Immunohistochemistry protocol for OCT3/4 were performed following (1) deparaffinization/rehydration (2) attractions antigen retrieval methods (3) Blocking buffers, Primary antibodies, incubation, secondary antibody (4) ABC complex (5) Staining with DAB
and AEC chromogen. Cells that express antibodies Oct3/4 are considered positive when it is expressed on the cell nucleus. We used primary antibody OCT 3/4 Santa Cruz Biotechnology, Santa Cruz USA Code- sc-5279 HIAR, Pre-treatment H2O2 for 5’ and biotin blocking, Dilutions and Incubation 1: 350 2 hours incubation at RT) Secondary antibody (Biotynilated) Horse anti-goat, AB Complex ABC- HRP, and Chromogen DAB

3. Result

We performed the test on 33 patients, newly diagnosed patients were 15 and the rest were paraffin block samples from patients retrieved previously which were consecutively performed with stem cell marker antibody OCT3/4.

<table>
<thead>
<tr>
<th>Table 1. Description of tissue differentiation status, histopathology analysis H&amp;E staining.</th>
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<tbody>
<tr>
<td><strong>Histology classification</strong></td>
</tr>
<tr>
<td>Dysgenetic gonad</td>
</tr>
<tr>
<td>UGT**</td>
</tr>
<tr>
<td>Testis</td>
</tr>
<tr>
<td>Ovary</td>
</tr>
<tr>
<td>Tube/fimbriae</td>
</tr>
<tr>
<td>Spermatic cord, Ductus</td>
</tr>
<tr>
<td>Others</td>
</tr>
<tr>
<td>* WHO histological classification of testis tumours, 2004** UGT: Undifferentiated gonadal tissue</td>
</tr>
</tbody>
</table>

Figure 1. The Hematoxylin and eosin staining. A. Lymphocytic and plasmacytic infiltrates in fibrous septa., cytogenetic result 46,XY/ 45 X, seminoma B. Hyalinized basement membrane, cytogenetic result: 46,XY, Gonadoblastoma (x200)

There were 6 samples which were excluded because did not meet criteria for inclusion, such as standar material for analysis, the finding of mixed liposarcome, testicular torsion, presence of neurofibroma plexiform, under and Leydic cell on ovary. Of the 33 samples, gonadal tissue is contained as many as 26 (1 UGT- Undifferentiated gonadal tissue, 1 dysgenetic gonads, 19 testes and 5 ovaries). Subsequently from the 26 examined samples, it was then identified for the presence of germ cells/precrusor. The result was as follow (table 2).

<table>
<thead>
<tr>
<th>Table 2. Identified malignancies of TGC using OCT3/4 staining</th>
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<tr>
<td><strong>Histology classification</strong></td>
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<tr>
<td>Seminoma Classical</td>
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<tr>
<td>Spermatocytic Seminoma</td>
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<tr>
<td>CIS**</td>
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<tr>
<td>Gonadoblastoma</td>
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<tr>
<td>Disgerminoma</td>
</tr>
<tr>
<td>Teratoma</td>
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<tr>
<td>Others</td>
</tr>
<tr>
<td>* WHO histological classification of testis tumours, 2004** CIS=Carcinoma in situ</td>
</tr>
</tbody>
</table>

Figure 2. The Immunocytochemistry staining OCT3/4 A. Presence of nuclear staining and lymphocytic infiltrates. in seminoma B. large primordial germ cells and gonadal stromal cells Gonadoblastoma

4. Discussion

Uncontrolled self-renewal has been proposed to be an important pathway in carcinogenesis [14]. The cancer stem cell theory since the establishment in 1997, indicated that the nature of frequency and treatment refracter orrelapsed tumor may be sustained by a subset of cancer cells with stem cell-like features that have the ability for self-renewal and pluripotency [15].

The presence of POU5F1/OCT-4 in GCT using immunohistochemistry was consistent with the previous finding. POU5F1/OCT-4 is an important factor for risk stratification. The protein was consistently detected in carcinoma in situ/gonadoblastoma, seminomas/germinoma/dysgerminoma, and embryonal carcinoma but not in the various types of differentiated nonseminomas [16]. The data indicate that pou5f1/POU5F1 functions as a master switch in differentiation by regulating cells that have, or can develop, pluripotent potential.

There were identified seminomal classical (n=2), spermatocytic seminoma (n=1), CIS (n=2), Gonadoblastoma (n=1), Disgerminoma (n=1), Teratoma (n=1), and others (n=4). Oct-4A is a transcription factor that n regulates the transcription of various genes and is expressed only in PSCs. POU5F1 binding sites have been identified in various genes, including fibroblast
promoter of the platelet-derived growth factor –α receptor. Oct-4B, on the other hand, is localized in cytoplasm of many non-pluripotent cell types and has no defined function as yet [17,18]. Besides embryonic stem (ES) cells, germ cells, primordial germ cells, and germ cell tumors [19,20], Oct-4 has also been reported in very small embryonic like stem cells (VSEL). Cell fate during development is defined by transcription factors that act as molecular switches to activate or repress specific gene expression programmes. The POU transcription factor Oct-3/4 (encoded by Pou5f1) is a candidate regulator in pluripotent and germline cells and is essential for the initial formation of a pluripotent founder cell population in the mammalian embryos) observed in various adult somatic tissues/organs in the body [21].

Recently, much progress has been made in the field of human spermatogonial stem cell (SSC) research with the successful derivation of ES cell-like colonies from adult. Pou5f1 binding sites have been identified in various genes, including fibroblast growth factor 4 and the 1.5-kb alternative promoter of the platelet-derived growth factor α receptor. The data indicate that pou5f1/Pou5f1 functions as a master switch in differentiation by regulating cells that have, or can develop, pluripotent potential.

OCT 3/4 is one of pluripotency-associated transcription factors other than NANOG, SOX2, and most recently LIN28 which has been identified as key regulators of pluripotency in mammalian embryonic and induced stem cells, and which are proven to be crucial for generation of the mouse germ-cell lineage [22]. Octamer 3/4 (OCT 3/4), also known as OCT 3, OCT 4 and Pou5f1 a member of the POU family, is considered to be an important stem cell marker and essential transcription factor during human embryogenesis [23]. In recent years, there have also been reports about the presence of Oct 3/4 in differentiated benign and malignant human cells [24].

Uncontrolled self-renewal is proposed to be an important mechanism in carcinogenesis. OCT3/4, is initially active as a maternal factor in the oocyte, also considered as one of the earliest transcription factors expressed in the embryo and recognized as fundamental in the maintenance of pluripotency and self-renewal in ESCs and in primordial germ cells. It is encoded by a homeobox-containing gene named Pou5f1 belonging to the family of Pit Oct Unc (POU) genes [25].

In this study, we detected the expression of OCT4 in 12 samples from 36 samples which were included in the initial course of the study. There was a wide range of histopathology changes in undescended testis. The transcription factor octamer-binding transforming factor 4 (Oct-4) is focus to the gene regulatory network responsible for self-renewal, pluripotency, and lineage commitment in embryonic stem (ES) cells and induced pluripotent stem cells (PSCs) [26].

The high expression of OCT-3/4 in fetal testes lasted only a few weeks, and subsequently both the number of positive cells and the intensity of staining rapidly decreased, and remained detectable only in a small proportion of gonocytes in the third trimester and the perinatal period. Thus, the expression of OCT-3/4 is normally no longer detectable in somatic tissues but is maintained at a high level in migrating primordial germ cells. OCT-3/4 was the first marker of embryonic stem cells associated with their pluripotency detected in human blastocytes [27].

In our study there were identified seminomal classical (n=2), spermatocytic seminoma (n=1), CIS (n=2), Gonadoblastoma (n=1), Disgerminoma (n=1), Teratoma (n=1), and others (n=4). The presence of CIS are large cells with distinct nucleoli, which in a typical arrangement of a single row with the common thickened basement membrane of seminiferous tubules with decreased diameters. In clinical setting CIS marker uses placental-like alkaline phosphatase [28]. In our study subject which collects samples from cryptorchidism cases with hypospadia and ambiguous Genitalia, it can be concluded that there is an increased of CIS and testicular cancer. This is line with previous clinical epidemiology of testicular germ cells [29].

TGCT are considered a paradigm of chemosensitive tumors due to its biology nature that in TGCT can be cured by chemotherapy even in advanced metastatic stages [30]. It was previously published that loss of OCT-4 causes a significant reduction of cisplatin hypersensitivity and was proposed to account for acquired cisplatin resistance in refractory tumors [31].

5. Conclusion

Taken all into account our observation might provide valuable information on the nature and behavior of tumors, leading to a new strategy for targeting CSCs and perhaps one step closer to the prevention of cancer recurrence and metastasis. OCT3/4 immunostaining is a promising and
useful tool in managing patients known to be at high risk for the development of invasive GCTs.

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Conflict of Interest

The authors report no conflicts of interest.

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