Omitted or Deficient - Hirschsprung Disease

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Congenital or acquired gastro-intestinal disorders comprise of a proportion of neonatal diseases. Hirschsprung disease, internal sphincter achalasia and pseudo intestinal obstruction are cogent neonatal disorders. Hirschsprung disease (HSCR) is a condition characterized by the complete absence of neuronal ganglion cells constituting the Meissner’s and Auerbach’s plexus within a variable portion of distal gastro-intestinal tract. The disorder frequently terminates in neonatal intestinal obstruction.

Hirschsprung disease is classified as

i) Short segment (aganglionosis restricted to the recto-sigmoid colon).

ii) Long segment (aganglionic segment extends proximal to the sigmoid colon).

iii) Total colonic aganglionosis. Majority (85%) of the instances are diagnosed in neonates.

Hirschsprung disease is contemplated as a neurocristopathy or a disorder of cells and tissues originating from neural crest cells. The malformation can be an isolated aspect or a multisystem manifestation with the evolution of extensive Hirschsprung disease.

Aganglionic segment of Hirschsprung disease comprises predominantly of distal rectum and a variable segment of contiguous proximal intestine. In an estimated 80% subjects, aganglionosis is confined to the recto-sigmoid colon (short segment disease), in roughly 15% to 20% individuals aganglionosis extends proximal to the sigmoid colon (long segment disease) and in approximately 5% neonates aganglionosis implicates the entire large intestine (total colonic aganglionosis). Infrequently, aganglionosis extends into the small bowel or proximally to envelop the entire bowel (total intestinal aganglionosis).

A preliminary diagnosis is preferred in order to avoid complications, especially enterocolitis which induces mortality [1,2].

Normal histology

Enteric nervous system is comprised of three nervous plexuses constituted by Auerbach’s or the myenteric plexus which is situated amidst dual layers of muscularis propria.

Henle’s plexus is situated superior to the circular layer of the muscularis propria and the site of superficial Meissner’s plexus is beneath the muscularis propria.

Normal nervous plexus is constituted by neurons (ganglion cells) and supporting glial cells.

Disease characteristics

Developmental disorder of Hirschsprung disease depicts an absence of intramural parasympathetic ganglion cells within submucosal and myenteric plexus of the rectum and variable distances of bowel proximal to the rectum. Approximate incidence of the condition is 1:

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5000 live births with an estimated 20% instances of comprehensive neonatal bowel obstruction and a mortality rate varying from 20% to 25% [2,3].

Hirschsprung disease is a disorder of neonates, infants and older paediatric patients and displays a male predominance with a male to female ration of 3 to 4:1.

Prevalence of short segment disease, cogitated in approximately 80% individuals with Hirschsprung disease, is four times frequent in males than females whereas equivalent gender specific instances are delineated in long segment Hirschsprung disease.

An estimated 48.7% subjects are detected within the first month of life, around 45.7% patients are discerned within first year of life, roughly 90.5% diagnosis are in newborns and approximately 54.3% neonates are discovered after first year of age.

According to genetic structure and ethnicity, specific groups display a higher incidence of Hirschsprung disease. Discernible family history is elucidated in around 40% instances particularly with total colonic aganglionosis and female subjects. Additionally, short segment aganglionosis displays a familial incidence and occurs preponderantly in females [3,4].

**Disease pathogenesis**

Anomalous cranio-caudal migration of vagal neural crest cells occurs during embryogenesis. Alternatively, the neural crest cells can degenerate following migration with consequential aganglionosis or hypoganglionosis of specified segment of the bowel. Allied Hirschsprung disease incorporates conditions with partial or integrated clinical signs of the disorder in concurrence with minimal quantities of ganglion cells. Symptomatic subjects in hypoganglionosis depict declining enumerated ganglion cells superior to the dentate line.

Zonal aganglionosis enunciates a bowel with limited aganglionosis enveloped with bowel segments of designated ganglion cells superior and inferior to the aganglionic segment. Aforesaid, infrequent condition can be engendered with ischemia, viral disease and immune-mediated injury.

Immature ganglion cells incorporate normal or enhanced quantification of morphologically abnormal ganglion cells with miniature nuclei and indistinct nucleoli. Immature ganglion cells are cogitated in neonates or premature infants and enhance physiologically with enhancing neonatal age. Immaturity can, however, persist [4,5].

**Clinical elucidation**

Classic variant of Hirschsprung disease is a short segment aganglionosis occurring in around 75% to 80% instances with implication of distal sigmoid colon and rectum with characteristic, enlarged, thickened and compact nerve filaments. Nerve filaments exceed 40 micron in diameter and are devoid of ganglion cells. Instances depicting minimal, miniature or lax, distinct from neurons, nerve filaments are indicative of adjunctive neuronal disorders and the diagnosis of Hirschsprung disease become questionable.

Long segment or total colonic aganglionosis demonstrates a reduced or almost absent nerve plexus.

Comprehensive clinical history and clinical correlation are essential to arrive at a cogent diagnosis.

The incriminated infant is frequently discerned within two months of neonatal life and exhibits symptoms consistent with impaired intestinal motility such as failure to pass meconium within the first forty eight hours of life (50% to 90%), constipation, emesis, abdominal pain, abdominal distension and occasional diarrhoea [5,6].

Hirschsprung disease is a cogent diagnosis in individuals with severe constipation since birth. Hirschsprung disease can engender enterocolitis and/or potentially lethal intestinal perforation.

Implicated bowel segment cogitated in Hirschsprung disease is aganglionic with tonic contractions. Aganglionic segment commences at the anus to extend proximally into the recto-sigmoid junction in an estimated 70% instances, within proximal colon in around 20% subjects and entire colonic length below < 10% neonates.
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Contingent to the evolution of enteric nervous system and absent ganglion cells, Hirschsprung disease engenders a functional obstruction in the distal colon [6,7].

**Tissue sampling**

A trans-rectal tissue specimen can be obtained under general anaesthesia or suction biopsy can be optimally utilized. Tissue specimens obtained from mucosa and submucosa predominantly evaluate the Meissner’s plexus although resection of deep seated tissue can demonstrate Henle’s plexus. Evaluation of specific tissue specimens is challenging. Appropriate quantities of rectal biopsies of adequate depth and magnitude are obtained and preserved in 10% formalin.

It is recommended to obtain tissue specimens at three or more levels with an indication of distance from the pectinate line. Orientation and specimen dissection is followed by a collection of an estimated 50 to 100 sections stained with haematoxylin and eosin. Evaluation of adequate tissue samples is suitable for a cogent differential diagnosis.

An adequate tissue specimen demonstrates the presence of mucosa and sub-mucosa and is collected at varying distances of one, two, three, four and five centimetres from the pectinate line. Magnitude of the specimen is necessitated to be betwixt 0.3 centimetres to 0.5 centimetres [3,4].

Micro-nodules within the mucosa or lymphoid aggregates configured in the sub-mucosa displacing the Meissner’s plexus are contemplated as inferior zones of sampling.

Sub-mucosa is identified and the biopsy with mucosa is placed horizontally on filter paper. Parallel incisions are created along the minor axis at a distance of 0.10 centimetres with a recommendation to avoid oblique incisions which contrive minimal sub-mucosa or specimens of only the “mucosal head” constituted of rectal mucosa or muscularis mucosa.

Sub-mucosal tissue is examined “For Innervation”. The block can be incised for two or three layers with an average of twelve to fifteen sections at each layer so that comprehensive quantities of sixty to one hundred and twenty slides can be attained per specimen which is contemplated as appropriate to discern aganglionosis. Supplementary assays such as relevant immune histochemical analysis is instituted [3,5].

Ideally, a tissue specimen is obtained one to two centimeters superior to the pectinate line. Specimens accessed inferior to aforesaid site or just superior to the anal sphincter can be devoid of ganglion cells even when representing a ganglionic segment of the bowel. Islands of squamous epithelial cells and muscle fibres originating from the sphincter can be elucidated. As such, aforementioned sites and tissue specimens are contemplated as insufficient or inadequate.

Appropriate rectal biopsy is constituted by a sub-mucosa, thickness of which is equivalent to the combined thickness of mucosa and muscularis propria. Lymphoid follicles situated in the sub-mucosa are contingent to an inadequate sampling. Neurons can be cogitated at the periphery of lymphoid follicles or impregnated within the muscularis mucosa, circumstances where aganglionosis is contemplated to be absent. Simplest diagnosis is one of a normal nerve fibre innervation of rectal mucosa enunciated at the mucosa/sub-mucosa junction [3,4].

**Histological elucidation**

Hirschsprung disease necessitates a demonstrable absence of sub-mucosal and myenteric ganglion cells from particular bowel segments for a cogent diagnosis. Characteristic histology of Hirschsprung disease depicts a complete absence of ganglion cells with a consequent augmentation of structural density of the nerve plexus. Glial cells are supplanted by Schwann cells, neuronal hypertrophy ensues and intestinal nerves recapitulate the appearance of peripheral nerves.

Histological techniques are appropriate and numerous in evaluating a rectal biopsy for discerning ganglion cells. Several serial sections of each tissue specimen are obtained and stained by haematoxylin and eosin which is a suitable diagnostic modality to discern Hirschsprung disease. Slides stained with haematoxylin and eosin are not always confirmatory as miniature, intermittently dispersed or neonatal
immature ganglion cells dispersed within the sub-mucosal region can lack appropriate elucidation. Innumerable sections require interpretation with skilled personnel to confer a diagnosis of a ganglionic/aganglionic bowel segment [7,8].

Preterm infants can occasionally display an innervation of immature ganglion cells. Aforesaid ganglion cells depict an aggregated, rosette like articulation with minimal neuropils and a morphology which is at variance from mature neuron cells. Immature ganglion cells are miniature, intensely stained bluish gray cells with eccentric nuclei, indistinct nucleoli with centric cytoplasm. Appropriate recognition and interpretation is crucial to prevent misdiagnosis.

Histochemical staining with acetyl cholinesterase (AChE) is an alternative technique. AChE staining can be employed upon formalin fixed, paraffin embedded sections. Aforesaid methodology aptly demonstrates AChE stained cholinergic nerve fibres within the lamina propria to indicate the presence of Hirschsprung disease, particularly enunciation of thick fibres appearing betwixt the crypts of lamina propria. Histochemical staining is indeterminate in subjects beneath 6 months of age or depicting short segment Hirschsprung disease.

Staining with AChE can be cogent with frozen section employing accurate staining protocols and freshly prepared reagents. AChE is considered as a preferred modality to diagnose Hirschsprung disease [8,9].

**Immune histochemistry**

Immune reactivity of ganglion cells to Bcl2 and neuron specific enolase (NSE) can be cogitated and approximately two to three levels of examination are sufficient to discern an aganglionic segment. Immune stains can be adapted to paraffin embedded serial sections from biopsies previously obtained, thus eliminating the requirement of additional specimens.

Relevant immune histochemical markers for discerning Hirschsprung disease are calretinin, neuron specific enolase (NSE), synaptophysin CD56, cathepsin D, vimentin, Bcl2, S100 protein and chromogranin A.

Calretinin is a vitamin D dependent calcium binding protein with a crucial role in calcium signaling, configuration and function of central nervous system. However, the precise function of calretinin remains obscure. Calretinin can discern stained neurons within the sub-mucosa, muscularis mucosa or the lamina propria.

Additionally, calretinin stains the ganglion cells intensely and Schwann cells minimally. Hirschsprung disease depicts a complete absence of staining within the entirety of bowel wall. Instances of mildly stained, enlarged nerve fibres with bowel segments devoid of ganglion cell staining can also be cogitated [9,10].

Calretinin depicts a superior immune reactivity to ganglion cells and is contemplated as a sensitive and suitable immune-stain for elucidating Hirschsprung disease.

Calretinin is immune reactive in normal muscularis mucosa, lamina propria and submucous nerve plexus where the Schwann cells are stained and nuclei of neurons are intensely accentuated. Aganglionic bowel demonstrates discretely immune reactive calretinin within nerve fibres with an absence of immune staining in the muscularis mucosa, lamina propria and nuclei of ganglion cells.

Synaptophysin is a synaptic vesicle glycoprotein exemplified in the pre-synaptic vesicles of neurons and neuro-endocrine cells. Antibodies to synaptophysin are reactive to the pre-synaptic vesicles of neurons cogitated in brain, spinal cord, neuromuscular junction, retina, vesicles of adrenal glands and islets of pancreas. Synaptophysin innervates the enteric bowel.

Synaptophysin demonstrates an intense staining of the myenteric plexus and is thus considered as a superior choice for detecting hypertrophic or hypotrophic modifications of extrinsic nerve fibres.

Synaptophysin reinforces the AChE stain in diagnosing Hirschsprung disease and allied disorders [10,11].

Chromogranin is a secretory protein situated in vesicles of neuroendocrine, endocrine, neuronal cells and sympathetic nerves. Immune staining with chromogranin A is beneficial chiefly in the recognition of neuro-epithelial and neuro-endocrine differentiation of normal and tumour tissue along with neuronal configuration of bowel and brain.
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In Hirschsprung disease chromogranin A depicts immune reactive adrenergic fibres within muscular layers of the aganglionic bowel segment. Concentration of chromogranin A as cogitated in the smooth muscle layer is elevated in the aganglionic bowel segment, in contrast to normal ganglionic bowel loops.

Instances depicting an absence of ganglion cells are not benefited with singular immune histochemical evaluation. Immune histochemical evaluation is preferably concurred with cogent haematoxylin and eosin stains [11,12].

Molecular modifications

A contemporary variant with elucidation of RET gene rs 2435357 is associated with Hirschsprung disease of diverse ethnicities. The variant appears in conjunction with a conserved transcriptional enhancer of RET and dismantles a SOX10 binding site within the MCS+9.7 with consequent declining enunciation of RET gene. Incriminated neonates depict an enhanced RET rs 2435357 risk allele [4,5].

Differential diagnosis

Intestinal neuronal dysplasia (IND) is a disorder which requires distinction from Hirschsprung disease. For diagnosis, an estimated twenty five sub-mucosal gangliaions are enumerated of which around 20% depict giant gangliaions with beyond >8 gangliaion cells, as elucidated in a paraffin embedded, haematoxylin and eosin stained cross section. Tissue specimens must be optimally obtained eight to ten centimetres superior to the pectinate line.

Thus, intestinal neuronal dysplasia (IND) remains a non-cogent diagnosis in infants below <1 year of age. Aforesaid disorder can concur with hypoganglionosis.

Hirschsprung disease necessitates a demarcation from specific disorders based on clinical symptoms and can be segregated with particular investigations of the disorder along with a tissue specimen of the ganglionic bowel obtained by suction [12,13].

Neonates with symptoms of intestinal obstruction can cogitate disorders such as

- Gastrointestinal atresia or malrotation or duplication of the gut.
- Meconium ileus secondary to cystic fibrosis.
- Conditions engendering ganglioneuromatosis such as multiple endocrine neoplasia -type 2b (men2b).
- Conditions accompanying anomalous enteric nervous system or musculature which is termed as chronic intestinal pseudo-obstruction including intestinal neuronal dysplasia (ind).
- Acquired varieties of severe constipation or intestinal obstruction can be secondary to maternal factors such as infection, alcohol ingestion or congenital hypothyroidism [3,4].

Mechanics of stem cell therapy

Stem cell therapy provides an additional therapeutic modality for the management of Hirschsprung disease.

Neural crest stem cells (NCSC) migrate from the branchial arches to bowel wall and are the predominant source of enteric nervous system (ENS) cells. The cells expand alongside vagal nerves to accumulate within distal gastrointestinal tract, the expansion being maximal during embryogenesis, in contrast to adjunctive cells. Enteric nervous system (ENS) cells are propagated from sacral neural crest in pelvic region.

Sacral neural crest cells arrive at the colon following evolution of vagal neural crest cells. However, sacral neural crest cells are incapable of preventing the development of Hirschsprung disease when devoid of a component of vagal neural crest cells. Miniscule aggregates of vagal neural crest cells can competently compose the colonic enteric nervous system [2,3].

Intestinal full thickness and submucosal biopsies of one centimetre magnitude or below obtained from infants can produce neurosphere like bodies (NLBs) on culture. The bodies are capable of migration and neuronal differentiation. Utilization of ganglionated bowel via extra-mucosal laproscopic biopsies from infants with Hirschsprung disease as derivatives of enteric nerve forming stem cells (ENSC) can prevent immune rejection in recipients.
Aganglionic bowel can also produce ENSC. Cultured neurons can be employed for autologous transplantation and differentiation of endogenous cells for treating aganglionosis. Ganglionic and aganglionic bowel of short segment and long segment Hirschsprung disease is a superior option for procuring ENSC, in contrast to bowel of total colonic and total intestinal aganglionosis [2,3].

Appropriate source of stem cells, stem cell transplantation and assessment of nerve cell migration, differentiation, connectivity and objective within the transplanted bowel can be evaluated. Stem cell transplantation can be applied in embryonic and postnatal disorders. Mesenchymal stem cells obtained from bone marrow can provide autologous stem cells for enteric nerve forming stem cell (ENSC) generation with the assistance of soluble growth factors.

Applicability of markers such as Ret and p75 are superior derivatives of enteric nervous system (ENS) stem cells.

Genetic and environmental concordance ensures genesis of enteric nervous system (ENS) and can enunciate the pathogenesis of Hirschsprung disease. Migratory ability of transplanted neuronal cells consists of a few millimetres along the gastro-intestinal length whereas the aganglionic segment is minimally of 10 centimetre magnitude. Glial cell line derived neurotrophic factor (GDNF) along with microenvironment of the recipient gut and migrating neuronal cells augment the migratory potential of transplanted cells. Such GDNF treated cells migrate twice the distance, enhance quantities of enteric nervous system (ENS) cells and magnitude of neurosphere following transplantation. Genetic mutation of GDNF gene and reduced elucidation of GDNF occurs in the aganglionic bowel [2,4].

Mutation of 16 genes are implicated in the evolution of Hirschsprung disease. RET is a major mutation. Contingent RET signaling within gastrointestinal immunity induces susceptible enterocolitis following conventional surgical intervention. Alteration of micro-environment of the recipient can prevent post therapy enterocolitis.

Genetic or dietary factors, nutrients and drugs modify the structure and function of enteric nervous system (ENS) in prenatal and postnatal period. Pathogenesis of Hirschsprung disease and irritable bowel syndrome can be implicated with aforesaid aspects; alteration of which can potentially prevent the occurrence of Hirschsprung disease.

Autologous stem cells provide an appropriate reservoir and prevent an immunological repercussion. Stem cells can be suitably injected throughout the gastrointestinal serosa via minimal access methodology. Intra-peritoneal and intravenous injections can be employed. However, viability and duration of implanted cells, incipient malignant conversion and the subcategory of Hirschsprung disease most benefitted from stem cell therapy require cogitation [2].

**Therapeutic options**

Alleviation of Hirschsprung disease comprises of surgical elimination of the aganglionic segment and an appropriate anastomosis betwixt the neuron innervated intestine and distal rectum. Previously, neonates with Hirschsprung disease were subjected to a colostomy with subsequent, definitive pull-through surgery at six to twelve month interval.

Currently, surgical eradication of the aganglionic intestinal segment is optimal therapy. Numerous definitive surgical procedures can be adopted such as a trans-abdominal endorectal pull through (Soave, Duhamel), trans-anal endorectal pull through (TEPT), trans-anal Swenson like and posterior neurectomy procedures. Aforesaid surgical options depict variable therapeutic outcomes. Currently, a primary pull-through such as trans-anal endorectal pull through (TEPT) is the preferable and frequently opted procedure [13,14].

**Therapeutic complications**

Enterocolitis occurs in 15% instances following specific surgery and can be discerned in 13% subjects sequential to endo-rectal pull through. Enterocolitis is not solely contingent to the category of surgical extermination.

Constipation appears with a frequency of 6% to 34%. Postoperative constipation depicts an incidence of 15% and is augmented with the adoption of posterior neurectomy (7.5%), probably due to lack of elimination of the aganglionic colon during the procedure of posterior neurectomy.

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Post-surgical morbidity is considerable, regardless of enhanced detection and therapeutic advancements. Morbid aspects comprise of persistent constipation, faecal incontinence, enterocolitis secondary to Hirschsprung disease and an exceptional, permanent stoma. Afore said morbidity arises on account of dysfunctional residual ganglionic segment, anomalous anal sphincter function, partially retained ganglionic distal bowel and complications arising from elimination of a portion or entire length of rectum.

Incidence of post-operative enterocolitis ranges from 4.6% to 54% along with reoccurring enterocolitis appearing at roughly 5%. Enterocolitis enhances post-operative mortality [12,14].

<table>
<thead>
<tr>
<th>Chromosomal defect</th>
<th>Phenotype</th>
<th>Genetic locus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Down's syndrome</td>
<td>ID, short stature, craniofacial features, CHD</td>
<td>Trisomy 21 (0.6% - 3%)</td>
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<tr>
<td>Deletion 10q11</td>
<td>ID, hypotonia</td>
<td>Del11.0q11.2 (RET)</td>
</tr>
<tr>
<td>Deletion 10q23</td>
<td>Isolated HSCR, rare recto-cutaneous fistula</td>
<td>Del11.0q23.1 (NRG3)</td>
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<tr>
<td>Deletion 13q</td>
<td>ID, craniofacial features, growth failure</td>
<td>Del11.3q22 (EDNRB)</td>
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<td>Deletion 2q22</td>
<td>ID, craniofacial features, seizures, microcephaly</td>
<td>Del12q22 (ZEB2)</td>
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<tr>
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<td>Del14p12 (PHOX2B)</td>
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<td>Deletions/duplications 17q21</td>
<td>ID, multiple congenital abnormalities</td>
<td>Del17q21/dup17q21-23 (unknown)</td>
</tr>
</tbody>
</table>

Table: Chromosome abnormalities with Hirschsprung disease [5].

CHD: Congenital Heart Disease; ID: Intellectual Disability.

**Figure 1:** Neuronal disarray with Schwann cells and absent ganglion cell sin Hirschsprung disease [15].

**Figure 2:** Thickened neuronal fibres stained with acetyl cholinesterase in Hirschsprung disease [16].
Figure 3: Demonstrable ret oncoprotein and absent ganglion cells in Hirschsprung disease [17].

Figure 4: Miniscule and aberrant sub-mucosal ganglion cells amidst muscularis propria in Hirschsprung disease [17].

Figure 5: Rectal mucosa devoid of ganglion cell plexus in Hirschsprung disease [18].

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Figure 6: Absence of ganglion cells in enteric plexus in Hirschsprung disease [19].

Figure 7: Transecting muscle cell layers with a lack of ganglion cells in Hirschsprung disease [20].

Figure 8: Hypertrophic nerve fibres highlighted with calretinin and devoid of ganglion cell plexus in Hirschsprung disease [21].

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**Figure 9:** Aberrant, enlarged neurons in aganglionic segment of Hirschsprung disease [15].

**Figure 10:** Serpentine, anomalous nerves and a lack of ganglion cells in enteric muscular coat of Hirschsprung disease [22].

**Figure 11:** Hirschsprung disease depicting a comparison of normal and aganglionic bowel segment [23].

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Figure 12: Toluidine blue stain highlighting aberrant, thickened nerves with absent ganglion cells in Hirschsprung disease [24].

Bibliography


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17. Image 3, 4 courtesy: Semantic Scholar.


20. Image 7 courtesy: Student source.

21. Image 8 courtesy: JCDR.


24. Image 12 courtesy: Pacific group of e journals.

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