Microsatellite Instability and Mismatch Repair Genes Variations Testing for the Identification of Lynch Syndrome among Palestinian Colorectal Cancer Patients

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The Annual Conference of Palestinian Society for Biological Sciences (ACPSBS)

September 4-5, 2021
Colorectal cancer

- Colorectal cancer (CRC) is an abnormal growth of cells that affect the colon or rectum.

(Ghorbanoghli et al., 2018)
Lynch Syndrome

- Lynch Syndrome (LS) is caused by a germline mutations in one of the mismatch repair (MMR) genes; 
  - MLH1, MSH2, MSH6, PMS2, and EPCAM.

- LS is inherited in an autosomal dominant pattern.  
  (Steinke et al., 2013)

- LS patients have 50-80% lifetime risk for developing CRC. 
  (Vasen et al., 1996)
Characteristics of CRC in LS patients

- Early onset
- Extra-colonic tumors
- Proximal right sided tumors
- Loss of MMR proteins
- MSI-H
- Poorly differentiated

(Lothe et al., 1993; Chen, Xu, & Liu, 2018)
Study objectives

• To describe the clinical and pathological characteristics of Palestinian CRC cases.

• To screen Palestinian CRC patients for Lynch Syndrome.

• To mount a platform for the genetic defects associated with Lynch syndrome.

• To describe the clinical and pathological characteristics of Lynch syndrome cases.

• To study the pedigree of the patients identified with Lynch syndrome.
Methodology
Study Population and Study Tools

176 pathologically confirmed CRC patients

Augusta Victoria Hospital (AVH) (n=126)

Beit-Jala Governmental Hospital (BJH) (n=50)

Pathology questionnaire

FFPE blocks

IHC

MSI testing

Disease characteristics

Family History

Family pedigree
Immunohistochemistry

176 FFPE block

MSH6 and PMS2

Intact expression

MLH1 and MSH2 (20 patients)

Loss of expression

MLH1 and MSH2
Microsatellite Instability Testing

- FFPE of tumor
- FFPE of normal tissue

DNA extraction

Touch down PCR

- Multiplex PCR (NR-21, NR-24, NR-27, BAT-25)
- Singleplex PCR (BAT-26)

ABI PRISM 3500 Genetic Analyzer

- NR-21 and BAT-25: 5HEX
- NR-24, NR-27, and BAT-26: 56-FAM

Fragment analysis

MSI-H  MSI-L  MSS
Results and Discussion
The Clinical Characteristics of CRC Patients

Gender

- Males: 52%
- Females: 48%

Age at diagnosis

- Mean age: μ = 57.5 ± 13.3 years
- Histogram showing distribution of ages

Regions

- Salfit: 1 (0.6%)
- Tulkarm: 9 (5.1%)
- Nablus: 8 (4.5%)
- West Bank: 20 (11.4%)
- Jericho: 17 (9.6%)
- Bethlehem: 6 (3.4%)
- Gaza: 27 (15.3%)
- Gaza Strip: 50 (28.4%)
- Other regions: 12 (6.8%)
- Total: 50 (100%)
Histological Location of Tumors and Disease progression in CRC patients

- **Histological location**
  - Proximal (12%) n=21
  - Distal (37%) n=65 (39%)
  - *Not specified in 12%

- **Disease stage**
  - Patients (%)
  - 0 1 2 28 49 54 43

- **Metastasis**
  - Stage VI
  - Others*
    - 34 (19.3%)
    - 10 (5.7%)
    - 9 (5.1%)
  - 3 (1.7%)
  - 2 (1.1%)
  - 4 (2.3%)
  - *Includes: breast, abdominal wall, bladder, pancreas, endometrium (n=1, 0.6%)
# Mismatch Repair Proteins’ Expression (IHC)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Intact</th>
<th>Loss of Expression</th>
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<tbody>
<tr>
<td><strong>PMS2</strong></td>
<td>164</td>
<td>12</td>
</tr>
<tr>
<td><strong>MSH6</strong></td>
<td>175</td>
<td>1</td>
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</table>

- **pMMR** 93%
- **dMMR**
  - PMS2+ MLH1 54% (n=7)
  - PMS2 46% (n=5)
  - MSH6+ MSH2 8% (n=1)

Intact expression
Loss of expression
Mismatch Repair Proteins’ Expression (IHC)

Intact expression of MSH6

Intact expression of PMS2
Mismatch Repair Proteins’ Expression (IHC)

Intact expression of MLH1

Loss of expression of MSH2

Loss of expression of MSH6

Intact expression of PMS2
Mismatch Repair Proteins’ Expression (IHC)

- Loss of expression of MLH1
- Intact expression of MSH2
- Intact expression of MSH6
- Loss of expression of PMS2
Characteristics of Patients with dMMR

Gender
- Male (n=4) 31%
- Female (n=9) 69%

Age at diagnosis: $\mu=57.2\pm18$ years

Tumor differentiation
- Poor (3)
- Moderate (8)
- Well (2)

Disease Stage
- I (3)
- II (2)
- III (3)
- IV (5)

Histological location
- Proximal (46.2%) (n=6)
- Distal (46.2%) (n=6)
- Rectum (7.6%) (n=1)
Microsatellite Instability Testing Results

NR-27: 73 bp, 83 bp
NR-21: 99 bp, 110 bp
NR-24: 115 bp, 123 bp
BAT-25: 140 bp, 151 bp

dMMR: 11
pMMR: 4

Bar Graph:
- Blue: MSI-H
- Red: MSI-L
- Orange: MSS

Percentage:
- 0%
- 10%
- 20%
- 30%
- 40%
- 50%
- 60%
- 70%
- 80%
- 90%

Range:
- 170-180 bp
MSS Fragment Analysis
Tumor vs. Normal Tissue

Tumor

Normal

Tumor

Normal
MSI-H Fragment Analysis
Tumor vs. Normal Tissue

Tumor

NR-27

NR-21

NR-24

BAT-25

Normal

Tumor

BAT-26

Normal
Families of nine of the 13 CRC patients with dMMR were interviewed, and four of them had previous history of cancer (44.4%).
Family Pedigrees

- The five other families did not report any case of cancer within their members (55.6%).
Limitations

• Regarding CRC patients:
  o Regional distribution
  o Advanced stage of disease

• Lack of full medical records.

• Inability to confirm diagnosis with Lynch Syndrome.
Conclusions

• Lynch Syndrome is estimated in 7.4% of CRC patients in Palestine.

• The majority of the patients had loss of PMS2 and MLH1 expression.

• dMMR patients were mostly females, with similar age to other CRC patients, and were diagnosed at an advanced stages of the disease.

• The majority of CRC patients with dMMR had MSI-H status.
Recommendations

• Further testing for dMMR patients.
  o To determine the exact cause of loss of gene expression

• Screen all newly diagnosed CRC patients for LS syndrome.

• Early screening for family members of CRC patients with dMMR.
  o Set policies and guidelines that encourage family members to screen for CRC from the age of 20-25 (every 1-2 years), and yearly after 40 years old.
Thank you
Acknowledgments

• Dr. Rania Abu Seir- Al-Quds University

• Beit-Jala Governmental Hospital-Ministry of Health
  o Dr. Mohammad Aqel
  o Pathology Laboratory
  o Dr. Nidal Al-Jibrini

• Augusta Victoria Hospital
  o Dr. Musa Hindiyeh
  o Dr. Marwan Qabajah
  o Pathology Laboratory
  o Molecular Laboratory
References