Detection of Colorectal Cancer Specific APC Deletion in Peripheral Blood Culture in Iraqi Population by FISH Technique

Haidar J. Muhammed2*, Mona Al-Terehi1, Ali H. Al-Saadi1

1University of Babylon, College of Science.
Al-Mustansiriyah University/ College of Science/ Department of Biology, Baghdad, Iraq.

*Corresponding Author: Haidar J. Muhammed

Abstract

Colorectal cancers (CRC) are classified as tumor phenotypes based on molecular profiles in which the most common defect is inactivation of adenomatous polyposis coli (APC) gene. This study included (20) patients male and female suffered from tumor of CRC range aged less than 20 and farther 60 years compared with (5) healthy individuals as a control. Samples collected from colonoscopy unit from Baghdad Teaching hospital and National Center Hospital for early detection of cancer related to Ministry of Iraqi Health. Deletion of APC gene was further verified on chromosomal level on metaphase spreads by peripheral blood lymphocytes (PBLs) culture so as value screening by using fluorescence in situ hybridization (FISH) technique. The results described the identification of classical familial adenomatous polyposis (FAP), attenuated familial adenomatous polyposis (AFAP) and sporadic colorectal cancer as in 58.3%, 25%, and 16.7 % respectively which were showed significant differences (P<0.01) compared with healthy control, so as a deletion of arm specific 5q of APC gene which showed significant differences (P<0.01). In conclusion of this study explained the APC gene alteration localized on 5q arm have an essential role in prognosis and prognostic of colorectal cancer.

Keywords: APC gene, 5q arm chromosome, AFAP and FAP gene in colorectal cancer.

Introduction

With a principle of fluorescence in situ hybridization (FISH) almost for the study of chromosome structure and function, as a combined of molecular and cytological approach, provide an intermediate degree of resolution between DNA analysis and chromosomal investigations especially in cancer [1-2]. Colorectal cancers are classified into specific tumor phenotypes based on molecular profiles which most common mutation is inactivation of Adenomatous polyposis coli (APC) gene [3-4], also known as deleted in polyposis 2.5 (DP2.5) which is a protein that in humans is encoded by the APC gene [1]. In the APC gene, mutations may result when APC does not have an inactivating mutation. These mutations can be inherited or arise sporadically often as a result of mutations in other genes that produce chromosomal instability. The risk of colorectal cancer by age 40 is almost 100% [5]. Mutation in (APC) gene results a familial adenomatous polyposis (FAP) which the patients with it have germ line mutations, percent with 95% mostly being (nononsense, point mutations/frame shift insertion or deletion nucleotide in a strand of DNA) mutations leading to premature stop codons causing loss of axinproien regulating (G protein) [6-7]. Most classical FAP patients have a family history of colorectal polyps and cancer; however 25-30% of them are actually without clinical or genetic evidence of FAP in family members [8-9]. The majority of patients polyps begin to develop during childhood, it was recognized that this can be partially explained by being the result of germ line mosaicism [10]. With two types of APC mutation gene; a classical a FAP is inherited as an autosomal dominant trait and results from a germline APC mutation that’s characterized by the presence of hundreds to thousands of colorectal adenomas of different sizes and attenuated familial adenomatous polyposis (AFAP) is mostly caused by specific APC mutations gene [11-12, 13-14-15] which is a less aggressive variant of FAP that is characterized by fewer colorectal adenomatous polyps (usually 10 to 100) as later age of adenoma appearance (mean age of polyp...
diagnosis is 44 years) and cancer (mean age 56 years). Clinically, it can be confusing as often there is mainly colonic involvement with polyps, and infrequent rectal involvement. Thus, it can be misdiagnosed as occurring in a patient with sporadic adenomas however, it can also occur in families with members having full clinical features of FAP. Although these individuals have a smaller polyp burden relative to classic FAP, they still have an increased risk of cancer that, in general occurs approximately 10-15 years later 25 [16-17-25]. The object of this study was to determine the diagnosis of colorectal cancer through APC of (5q) arm deletion gene by detection of FISH technique.

Materials and Methods

Cytogenetic analysis method of Rooney and Czepulkowsk, through blood culture was performed in (20) patients male/female aged with less than 20 years and further 60 years which suffered from CRC with two types of progressive polyps classical FAP and attenuated AFAP compared with [5] normal healthy. The Blood samples collected from colonoscopy unit of Baghdad Teaching Hospital and National Center Hospital for early detection of cancer related to Ministry of Iraqi health.

The samples which were carried out by cytogenetic test selected on an increasing percent of aberrant chromosomal of aneuploidy and polyplody through detection of cytogenetic aberration through the test which was done for these patients. So as Fluorescence in situ hybridization (FISH) technique for arm specific probe (ASP 5q arm) was used on metaphase spread to screen alterations and defects of deletion on chromosome 5q arm for the samples as mentioned in the leaflet kit of this marker. Patient's blood samples were tested to metaphase blood culture [18].After (71.5) hours of culturing of peripheral blood cells (PBC), cytogenetic aberration exam was done to establish the diagnoses and recognize the metaphase spreads of aberration chromosomes which was stained by Giemsa dye and screening was done under high power magnifications (100 X) [18- 19-28].

Results and discussion

The current study tested the association deletion of chromosome 5q arms as marked with a sign (+) that includes the area of APC gene in a prognosis of tumor in patients with CRC as shown in tables [1-2].

The data explained 60% (12 out of 20) of all patients which have a deletion consisted by (20%- 35%) male to female with a distribution aged represented by less (20, 21- 40 and 60) years. While 5% have two positive signs (++) whom was a male patient, aged less than 60 years as shown in the same tables. These patients have APC gene deletion marked with (+). The sign (+) represents a deletion in one arm of 5 chromosome and the two signs (++) represented of double deletion in two arms of two 5 chromosomes which observed by (screened and or/ not) greenish color markers in a fluorescence microscope as appeared in a field and represented by table [2] of the frequency of the patients and healthy. The results compared with the healthy subjects which have appeared with two arms of 5 chromosomes as explained by figures [1-2].

A current study showed most of the deletions were large. The whole deletion area of 5q arm which content of APC gene was verified on chromosome level by using FISH technique on metaphase spreads as shown in figure [3] and these data corresponding with a previous studies [18-19-26]. The patients suffered from three types of polyps represented by familial adenomatous polyposis (FAP) and attenuated familial adenomatous polyposis (AFAP) so as through progression of polyps to adenocarcinoma. So as the tested samples selected on an increasing percent of aberrant chromosomes (Aneuploidy & Polyplody) through detection aberration of cytogenetic test which done for their patients with a less number of other aberrant.

Table 1: Positive and negative deletion of (5q) arm chromosome in patients with tumor and CRC

<table>
<thead>
<tr>
<th>Patients samples/20</th>
<th>Age group</th>
<th>Count</th>
<th>% of Total</th>
<th>Total</th>
<th>Chi-square (χ²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As 5q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-ve</td>
<td>0-20 years</td>
<td>0</td>
<td>0.0%</td>
<td>3</td>
<td>8.451 **</td>
</tr>
<tr>
<td>++</td>
<td>21-40 years</td>
<td>1,1</td>
<td>5.0%</td>
<td>4</td>
<td>7.393 **</td>
</tr>
<tr>
<td>++</td>
<td>41-60 years</td>
<td>1</td>
<td>5.0%</td>
<td>1</td>
<td>1.174 NS</td>
</tr>
<tr>
<td>++</td>
<td>&gt; 60 years</td>
<td>1</td>
<td>5.0%</td>
<td>7</td>
<td>8.744 **</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1</td>
<td>5.0%</td>
<td>3</td>
<td>5.0% (P&lt;0.05)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1</td>
<td>5.0%</td>
<td>3</td>
<td>5.0% (P&lt;0.01)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1</td>
<td>5.0%</td>
<td>3</td>
<td>5.0% (NS)</td>
</tr>
</tbody>
</table>

* (P<0.05), ** (P<0.01), NS: Non-significant.
Table 2: Frequency arm specific deletion chromosome 5q arm in tumor and CRC in patients compare with healthy subjects of a study

<table>
<thead>
<tr>
<th>Patients samples/ 20</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>As 5q</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-ve</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>+</td>
<td>11</td>
<td>44</td>
</tr>
<tr>
<td>++</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>100.0</td>
</tr>
<tr>
<td>Chi-square (χ²)</td>
<td>----</td>
<td>9.026 **</td>
</tr>
</tbody>
</table>

** (P≤0.01).

Figure 1: Panel showed two alleles of chromosome specific 5q arm of healthy subjects screened by FISH technique. The panel showed no deletion in chromosome 5q arm.

Figure 2: Panel showed deletion of single allele of 5 chromosome in patient with CRC screened by FISH technique.

Figure 3: Deletion of chromosome 5q (+) arm in one copy and deleted the other allele copy in patients with CRC by FISH technique.
These results corresponding with the previous studies which denoted that colorectal cancer like most human cancers is characterized by genomic instability which have allelic imbalance at a number of chromosomal loci, including 5q, 8p, 17p, and 18q [21- 22- 23-24-27].

Conclusion

References


