1. Introduction

The most common cancer in males is the prostate [1] cancer whereas the most common cancer and malignancy in female is the breast cancer [2–5]. Among cancers, colorectal and lung cancers are of the most widespread cancers in both genders [1]. So colorectal cancer has a strategic importance for governments. Although other cancers like ovarian and endometrial cancers are also prevalent in women [6,7], but the strategic importance of colorectal cancers is higher mortality and affecting both genders.

Colorectal disease like Crohn’s disease and ulcerative colitis has very strategic importance from the epidemiological points of view [8] as well as colorectal cancers. Colorectal cancer is one of the most common types of cancers, particularly in developed countries and it is defined as growth and spread of cancer cells in colon or rectum [9]. This disease usually has no specific symptom and often diagnose in latest and dangerous stages of the disease. Most important symptoms are blood existence in stool, weight loss and permanent fatigue [10,11]. The cause of getting this cancer is related to lifestyle, age and gender (men will getting this cancer more than women) because of obesity, alcohol consumption, high consumption of red meat, absence of fibers in diet, physical inactivity and especially tobacco abuse [12–14]. Increasing age also has a significant impact on the risk of this cancer so that a lot of people over 70 years of age in Western societies gett adenoma and adenoma increases the possibility of getting this cancer [15,16]. This disease has several stages. In the first stage, cancer cells are in inner layer of colon or rectum, in the next stage the cancer spreads in muscular layer of colon or rectum, thereafter in a next stage, the cancer enter to a few lymph nodes in the same area and after that in another stage metastasis occurs and the cancer spreads into other tissues and body areas; at this stage the disease is usually not curable [17–19]. Most colorectal cancers originate from the benign adenomas that forms in colon. Molecular and genetic studies indicate that about 70% of colorectal cancers arise by mutation and inactivation of the gene adenomatous polyposis coli (APC) and other tumors arising as a result of activating mutations in the genes that producing beta-catenin and axin [16,20–22]. Also other genetic studies have shown that this cancer usually arises due to the mutations that cause instability of some chromosomes and change the structure of these chromosomes. These chromosomal changes cause not to produce an enough number of copies of the tumor suppressors like APC and P53 [23–28]. Other than tumor suppressor genes, this and some other cancers can be affected by proto-oncogenes like the ubiquitin-like with PHD and ring-finger domains 1 (UHRF1) [29]. Other researches have indicated that mutation in KRAS and epidermal growth factor (EGF) signaling pathways have an important role in getting this cancer [30,31].

Immune system responses have important role in controlling and preventing development of colorectal cancers [26]. Colorectal cancer...
cells have several antigens which recognized by immune system. Among these antigens, Carcinoembryonic antigen (CEA) is the most studied [32,33]. In addition, several neo-antigens were detected in these tumor cells. These neo-antigens are the cause of high levels of lymphocytes infiltrating to the tumor cells [23]; so the cancer progression can be diagnosed from the level of lymphocyte infiltration. Since generally the cancerous cells fail to express human leukocyte antigen (HLA) class I and therefore bypass cytotoxic T lymphocytes, natural killer-cells (NKs) as important components of the innate immune system, play a key role in this condition [34]. Killer cell immunoglobulin-like receptors (KIRs) (also called as CD158) are polymorphic glycoproteins expressed on cell surface of NKs and T cell subsets [35]. KIR gene family is highly polymorphic and its genomic diversity is achieved through differences in gene content as well as allelic polymorphism [36,37]. Of course the most polymorphic loci in human genome is HLA [38] which the molecules of its, are in direct contact with KIR molecules. Hereby we intend to investigate the role of the KIR genes in colorectal cancer as a meta-analysis to find the association of different genes of KIR and susceptibility to be affected by colorectal cancer.

2. Material and methods

The present study is a meta-analysis which approximately covers all the original studies on this topic done before. We searched in databases such as google scholar, science direct and Pubmed. Totally six papers were found that four of them had same protocols.

| Study name | Odds ratio | Statistics for each study | Odds ratio and 95% CI |
|------------|------------|---------------------------|-----------------------|
|            | Lower limit| Upper limit               | Z-Value | p-Value |
| Middleton et al. | 1/042 | 0/619 | 1/752 | 0/154 | 0/878 |
| Beksa et al. | 0/661 | 0/146 | 1/051 | 1/751 | 0/080 |
| Al Omar et al. | 0/856 | 0/631 | 1/162 | 0/995 |
|              |            |                          | 0/01 |             |

**Favours A**

**Favours B**

![Fig. 1.](image) Colorectal cancer is not statistically affected or protected by the KIR2DL1. Left side (the favours A) shows protecting effect in all figures.

### Table 1

| KIR genes | Inhibitory KIRs | Activating KIRs | Pseudo genes |
|-----------|----------------|----------------|--------------|
| 2DL1      | 1              | 1              |              |
| 2DL2      | 1              | 1              |              |
| 2DL3      | 1              | 1              |              |
| 2DL4      | 1              | 1              |              |
| 2DL5      | 1              | 1              |              |
| 2DL6      | 1              | 1              |              |
| 2DL7      | 1              | 1              |              |
| 2DL8      | 1              | 1              |              |
| 2DL9      | 1              | 1              |              |
| 2DL10     | 1              | 1              |              |
| 2DS1      | 1              | 1              |              |
| 2DS2      | 1              | 1              |              |
| 2DS3      | 1              | 1              |              |
| 2DS4      | 1              | 1              |              |
| 2DS5      | 1              | 1              |              |
| 2DS6      | 1              | 1              |              |
| 2DS7      | 1              | 1              |              |
| 2DS8      | 1              | 1              |              |
| 2DS9      | 1              | 1              |              |
| 2DS10     | 1              | 1              |              |
| 3DS1      | 1              | 1              |              |
| 3DS2      | 1              | 1              |              |
| 3DS3      | 1              | 1              |              |
| 3DS4      | 1              | 1              |              |
| 3DS5      | 1              | 1              |              |
| 3DS6      | 1              | 1              |              |
| 3DS7      | 1              | 1              |              |
| 3DS8      | 1              | 1              |              |
| 3DS9      | 1              | 1              |              |
| 3DS10     | 1              | 1              |              |

### Table 2

Data and meta-analysis. The ED stands for effective direction and the negative and positive each one respectively means protective effect and risk factor.

| Gene | Middleton et al. [58] Cancer N = 90 Control N = 100 | Beksa et al. [60] Cancer N = 87 Control N = 154 | Al Omar et al. [61] Cancer N = 52 Control N = 70 | Kim et al. [57] Cancer N = 241 Control N = 159 | Meta-analysis P-value (ED) |
|------|-----------------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|---------------------------|
| 2DL1 | 86                                                  | 94                                            | 77                                            | 147                                           | 2DL1 51 70 241 159 0.32   |
| 2DL2 | 61                                                  | 57                                            | 68                                            | 103                                           | 2DL2 47 55 27 20 0.07     |
| 2DL3 | 69                                                  | 85                                            | 48                                            | 91                                            | 2DL3 48 96 56 97 0.59     |
| 2DL4 | 52                                                  | 32                                            | 52                                            | 32                                            | 2DL4 45 53 241 159 0.99   |
| 2DL5 | 50                                                  | 25                                            | 50                                            | 25                                            | 2DL5 45 32 241 159 0.45   |
| 2DL6 | 36                                                  | 39                                            | 52                                            | 52                                            | 2DL6 45 53 241 159 0.45   |
| 2DL7 | 34                                                  | 34                                            | 34                                            | 34                                            | 2DL7 34 34 241 159 0.45   |
| 2DL8 | 32                                                  | 32                                            | 32                                            | 32                                            | 2DL8 34 32 241 159 0.45   |
| 2DL9 | 30                                                  | 30                                            | 30                                            | 30                                            | 2DL9 34 32 241 159 0.45   |
| 2DL10 | 29                                                  | 29                                            | 29                                            | 29                                            | 2DL10 34 32 241 159 0.45  |
| 2DS1 | 36                                                  | 36                                            | 40                                            | 36                                            | 2DS1 43 43 25 106 0.28   |
| 2DS2 | 36                                                  | 36                                            | 54                                            | 36                                            | 2DS2 46 46 50 31 0.09    |
| 2DS3 | 34                                                  | 34                                            | 52                                            | 34                                            | 2DS3 40 40 40 32 0.81    |
| 2DS4 | 32                                                  | 32                                            | 70                                            | 32                                            | 2DS4 51 51 70 229 0.41   |
| 2DS5 | 26                                                  | 26                                            | 51                                            | 51                                            | 2DS5 35 35 22 80 0.0001   |
| 2DS6 | 34                                                  | 34                                            | 52                                            | 34                                            | 2DS6 43 43 16 99 0.18    |
| 2DS7 | 32                                                  | 32                                            | 76                                            | 32                                            | 2DS7 43 43 16 99 0.18    |
| 2DS8 | 30                                                  | 30                                            | 76                                            | 30                                            | 2DS8 43 43 16 99 0.18    |
| 2DS9 | 28                                                  | 28                                            | 76                                            | 28                                            | 2DS9 43 43 16 99 0.18    |
| 2DS10 | 26                                                  | 26                                            | 76                                            | 26                                            | 2DS10 43 43 16 99 0.18   |
| 3DS1 | 34                                                  | 34                                            | 35                                            | 76                                            | 3DS1 43 43 16 99 0.18    |
| 3DS2 | 34                                                  | 34                                            | 35                                            | 76                                            | 3DS2 43 43 16 99 0.18    |
| 3DS3 | 34                                                  | 34                                            | 35                                            | 76                                            | 3DS3 43 43 16 99 0.18    |
| 3DS4 | 34                                                  | 34                                            | 35                                            | 76                                            | 3DS4 43 43 16 99 0.18    |
| 3DS5 | 34                                                  | 34                                            | 35                                            | 76                                            | 3DS5 43 43 16 99 0.18    |
| 3DS6 | 34                                                  | 34                                            | 35                                            | 76                                            | 3DS6 43 43 16 99 0.18    |
| 3DS7 | 34                                                  | 34                                            | 35                                            | 76                                            | 3DS7 43 43 16 99 0.18    |
| 3DS8 | 34                                                  | 34                                            | 35                                            | 76                                            | 3DS8 43 43 16 99 0.18    |
| 3DS9 | 34                                                  | 34                                            | 35                                            | 76                                            | 3DS9 43 43 16 99 0.18    |
| 3DS10 | 34                                                  | 34                                            | 35                                            | 76                                            | 3DS10 43 43 16 99 0.18   |

**Significance at P < 0.05 level.**
The overall population of this four studies consists of 953 individuals (470 individuals with colorectal cancer and 483 individuals in control groups). The test chi-squared 2 multiplied by 2 with Yate's correction was used to assay each gene separately. Then the results were imported into the software comprehensive meta-analysis version 2. The 5 genes 2DL4, 3DL2, 3DL3, 2DP1 and 3DP1 were excluded from test because of their persistence in all participants of the both groups.

3. Results

3.1. About KIR

Depending on the number of extracellular immunoglobulin domains, KIRs are divided into two distinct groups (2D or 3D). Two types of KIR, i.e. inhibitory and activating, have been distinguished based on length of the intracellular domain. Inhibitory KIRs (iKIRs) are characterized by a long intra-cytoplasmic tail (denoted by an ‘L’ in their name) and presence of at least one immunoreceptor tyrosine-based inhibitory motif (ITIM). Activating KIR (aKIR) are characterized by a short intra-cytoplasmic tail (denoted by an ‘S’ in their name) and the absence of ITIM [37].

Up to now, fourteen distinct types of KIR have been identified in the human genome [39]. NKs are a subset of lymphocytes comprising around 10–15% of total lymphocytes in peripheral blood [40]. NKs principally contribute to innate immunity and also adaptive immune responses by killing the targeted cells of theirs and production of a variety of cytokines and chemokines [37]. Overall, upon interaction with their ligands which are usually HLA class I, KIR provide inhibitory

| Study name         | Odds ratio | Lower limit | Upper limit | Z-Value | p-Value |
|--------------------|------------|-------------|-------------|---------|---------|
| Middleton et al    | 1/440      | 0/854       | 2/428       | 1.367   | 0/171   |
| Beksc et al        | 1/493      | 0/939       | 2/374       | 1.695   | 0/090   |
| Al Omar et al      | 1/642      | 0/851       | 3/171       | 1.478   | 0/139   |
| Kim et al          | 0/953      | 0/867       | 1/362       | 0/264   | 0/792   |
| 1/240              | 0/962      | 1/564       | 0/810       | 0/070   |

Fig. 2. Colorectal cancer is not statistically affected or protected by the KIR2DL2.

| Study name         | Odds ratio | Lower limit | Upper limit | Z-Value | p-Value |
|--------------------|------------|-------------|-------------|---------|---------|
| Middleton et al    | 0/711      | 0/423       | 1/203       | 1.267   | 0/205   |
| Al Omar et al      | 1/722      | 0/891       | 3/329       | 1.617   | 0/106   |
| 1/002              | 0/966      | 1/569       | 0/011       | 0/991   |

Fig. 3. Colorectal cancer is not statistically affected or protected by the KIR2DL3.

| Study name         | Odds ratio | Lower limit | Upper limit | Z-Value | p-Value |
|--------------------|------------|-------------|-------------|---------|---------|
| Middleton et al    | 1/095      | 0/651       | 1/843       | 0/344   | 0/731   |
| Al Omar et al      | 1/515      | 0/786       | 2/920       | 1/241   | 0/214   |
| Kim et al          | 1/277      | 0/893       | 1/826       | 1/340   | 0/180   |
| 1/262              | 0/964      | 1/651       | 1/694       | 0/090   |

Fig. 4. Colorectal cancer is not statistically affected or protected by the KIR2DL5.
or activating signals to regulate the activity of NKs, which contributes to pathogenesis of diverse kinds of diseases [41,42]. Different compounds of KIR-HLA genotypes can induce different thresholds of activation in NK family and such genotypic variations have been found to be associated with a number of human diseases and complications including viral infections, autoimmune disorders and cancers [43] as well as reproduction abnormalities [44,45]. The KIR gene cluster on chromosome 19q13.4 within the leukocyte receptor complex (LRC) consists of a centromeric and telomeric region [46]. So far, 14 KIR genes and 2 pseudogenes have been described [47] (Table 1). Seven genes of KIR3DL1-3, KIR2DL1-3 and KIR2DL5 encode for the inhibitory KIR (iKIR), six genes of KIR3DS1 and KIR2DS1-5 encode for activating KIRs (aKIR), one gene encodes for KIR2DL4 with both inhibitory and activating functions, but more of inhibitory, and two genes of KIR2DP1 and KIR3DP1 are pseudogenes that do not encode a functional KIR molecule [47].

| Study name           | Odds ratio | Lower limit | Upper limit | Z-Value | p-Value |
|----------------------|------------|-------------|-------------|---------|---------|
| Middleton et al      | 1/1000     | 0.595       | 1.682       | 0.000   | 1.000   |
| Al Omar et al        | 1/211      | 0.631       | 2.327       | 0.576   | 0.565   |
| Kim et al            | 0/786      | 0.650       | 1.124       | 1/318   | 0/188   |

Odds ratio and 95% CI

|       |       |       |       |       | |
|-------|-------|-------|-------|-------|-------|
|      0/01 | 0/1 | 1 | 10 | 100 | |

Favours A  Favours B

Fig. 5. Colorectal cancer is not statistically affected or protected by the KIR3DL1.

Fig. 6. KIR2DS1. The arrowed population in for the study of Al Omar et al.

Fig. 7. KIR2DS1 meta-graph, before exclusion of Al Omar’s et al. study.
About NK subsets, we have mainly CD16\textsuperscript{+} CD56\textsuperscript{dim} and CD16\textsuperscript{−} CD56\textsuperscript{bright}; the dim form has more cytotoxic capacity called as "cytotoxic NK" and the bright form contributes in secretion of inflammatory cytokines called as "immune-regulatory NK". Both of them express KIR, but the dim form express more.\cite{35,37,44,46,48–52}.

The KIR gene cluster is flanked by KIR3DL3 at centromeric end and KIR3DL2 at telomeric end; both of which are present on virtually all haplotypes. Two groups of KIR haplotypes have been defined on the basis of gene content and are termed as haplotypes A and B. The A haplotypes are uniform in terms of gene content and the most prevalent form of them is composed of five inhibitory genes (KIR2DL1, 2DL3, 3DL1, 3DL2 and 3DL3), one activating gene (KIR2DS4), and the KIR2DL4 which may have both inhibitory and activating capacity. Interestingly, many A haplotypes possess null variants of both KIR2DS4 and KIR2DL4 that are not expressed on the cell surface. Thus these haplotypes technically possess no functional aKIR gene. The B haplotypes contain variable numbers of activating and inhibitory genes and are the primary contributors to the extraordinary differences in KIR gene profiles observed in distinct ethnic populations across the world. The interaction of inhibitory KIR with HLA class I as their ligands, triggers the signals that turn off

### Meta Analysis

| Study name      | Statistics for each study | Odds ratio and 95% CI |
|-----------------|----------------------------|-----------------------|
| Middleton et al | 1/038                      | 0/617 – 1/746          |
| Beksac et al    | 0/940                      | 0/593 – 1/450          |
| Kim et al       | 1/349                      | 0/943 – 1/929          |

---

**Fig. 8.** KIR2DS1 meta-graph, after exclusion of Al Omar’s et al. study.

---

### Meta Analysis

| Study name      | Statistics for each study | Odds ratio and 95% CI |
|-----------------|----------------------------|-----------------------|
| Middleton et al | 1/388                      | 0/824 – 2/340         |
| Beksac et al    | 0/866                      | 0/546 – 1/373         |
| Al Omar et al   | 1/984                      | 1/022 – 3/851         |
| Kim et al       | 0/740                      | 0/517 – 1/059         |

---

**Fig. 9.** Colorectal cancer is not statistically affected or protected by the KIR2DS2.

---

### Meta Analysis

| Study name      | Statistics for each study | Odds ratio and 95% CI |
|-----------------|----------------------------|-----------------------|
| Middleton et al | 0/974                      | 0/579 – 1/638         |
| Beksac et al    | 1/174                      | 0/740 – 1/862         |
| Al Omar et al   | 2/000                      | 1/030 – 3/834         |
| Kim et al       | 0/803                      | 0/562 – 1/148         |

---

**Fig. 10.** Colorectal cancer is not statistically affected or protected by the KIR2DS3.
the NKs. Therefore, by expressing HLA-A, -B and -C molecules, healthy cells are protected against NK lysis. Down-regulation of HLA class I expression due to tumor transformation or viral infection permits NKs to lyse these unhealthy targeted cells of theirs, a phenomenon first described as the "missing-self" hypothesis. Thus the compound KIR-HLA genotypes that lead to lower inhibition and higher activation appear to be beneficial in resistance to viral infections and cancers. On the other hand, these dominant activating genotypes may constitute a risk for susceptibility to autoimmune and inflammatory diseases [43].

3.2 KIR and colorectal cancer

As a general rule NKs play a key role against the cancerous cells escaped from toxic activity of T cells. So the KIRs expressed on surface of NKs become important. We know that the interaction KIR-HLA has two sides of KIR and HLA. For example overexpression of HLA-E on surface of colorectal cancer cells can result in inhibition of NKs [53] and such patients have better survival from the disease [54]; of course the receptor of HLA-E is CD94/NKG2a [55,56]. KIR2DL1 and 2DS1 interact
with HLA-C2, and KIR2DL2, 2DL3 and 2DS2 interact with HLA-C1 [57]; so every mathematical predict do not occur. Although at the first glance it seems that inhibitory types of each or both sides may be associated with the more susceptibility, but this rule is not true for such inflammation-based cancers. The whys and therefore of this paradox, is not clear; however there are some good data in study of Middleton et al. [58] about loss of HLA expression in colorectal cancer cells giving us insight. Also role of allograft inflammatory factor 1 (Alf1) shows the inflammation base of this disease [59].

Although the most inhibitory effect is attributed to the interaction KIR2DL1-HLA-C2 [57], but none of our analyzed studies do not show any significant protective effect. Of course we reanalyzed previous articles’ data with Yate’s correction; so the relation of some genes in some studies were significant in the study of theirs without Yate’s correction. For example Beksac et al. [60] had been found a significant protective effect for KIR2DL1 as we did not found so (Fig. 1).

Among the four studies analyzed by us (Table 2), the three genes 2DL4, 3DL2 and 3DS1, and both pseudogenes were present in approximately all participants of the studies, so these genes were excluded from our meta-analyses. Among the other genes, at first, the meta-analyses showed a significant association for the genes 2DS1, 3DS1 and 2DS5 (Figs. 1–15); but because of high odds ratio of study of Al Omar et al. in Saudi Arabia [61] for the genes 2DS1 and 3DS1 (Figs. 6, 13), we were supposed to exclude it from the analysis of these two genes. After the exclusion, only association of KIR2DS5 remained significant (Figs. 7, 8, 12, 14, 15).

Another justification for this paradox is different basis of different cancers. As we published before, breast cancer is affected by KIR2DL2 which is an inhibitory gene [62]. In verse, colorectal cancer is affected by an activating KIR as we found in the present meta-analysis. Although at the first glance it seems that inhibitory interactions of KIR-HLA may be associated with the more susceptibility to colorectal cancer because of this fact that NKs play a key role against the cancerous cells escaped from toxic activity of T cells because of their loss of HLA expression, but this rule is not true for such inflammation-based cancers.

Other than the justification above, another justification is that the ligands of activating KIRs are not necessarily HLAs; rather, they are still unknown [36,53]. This, can be pointed out as the limitation of the previous studies.

4. Conclusion

Finally we concluded that colorectal cancer is affected by KIR2DS5 and also there were no protecting gene. This result shows the inflammatory basis of this cancer. In other words, in contrast to leukemia and blood cancers, colorectal cancers seem to be affected by hyper activity of natural killer-cells (NKs). Whys and therefore of this paradox, is suggested to be investigated further.

Conflict of interest

We declare that there is no conflict of interest.
class I characterised bladder, colorectal and laryngeal tumours. Tissue Antigens 69 (2007) 220–226.

[59] C. Sanchez, C. Baier, J.G. Colle, R. Chelbi, P. Ribet, T. Le Treut, J. Imbert, G. Sébahoun, G. Venton, R.T. Costello, Natural killer cells in patients with polycythemia vera. Hum. Immunol. 76 (2015) 644–650.

[60] K. Beksac, M. Beksac, K. Dalva, E. Karaagaoglu, M.B. Tirnakiz, Impact of “Killer immunoglobulin-like receptor/ligand” genotypes on outcome following surgery among patients with colorectal cancer: activating KIRs are associated with long-term disease free survival. PLoS One 10 (2015), e0132526.

[61] S.Y. Al Omar, L. Mansour, J.A. Dar, S. Alwasel, A. Alkhuriji, M. Arafah, O. Al Obeed, S. Christmas, The relationship between killer cell immunoglobulin-like receptors and HLA-C polymorphisms in colorectal cancer in a Saudi population. Genet. Test. Mol. Biomarkers 19 (2015) 617–622.

[62] B. Shayanrad, S.A.Y. Ahmadi, F. Shahsavar, Breast cancer is protected by the KIR gene 2DL1 and affected by 2DL2: a systematic review. Der Pharmacia Lettre 8 (2016) 22–25.