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POLYMORPHIC SIGNATURES OF IL-1RA GENE AND ITS ASSOCIATION WITH CANCER SUSCEPTIBILITY AND PROGRESSION IN SOUTHERN INDIA

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Abstract

Cancer remains one of the leading causes of mortality worldwide, with genetic and immunological factors playing a central role in its onset and progression. The interleukin-1 receptor antagonist (*IL-1RA*) gene, an immune-regulatory gene, influences inflammation-driven tomourigenesis and angiogenesis. Variations in *IL-1RA* may therefore serve as potential biomarkers of cancer susceptibility. This study investigated the association of *IL-1RA* variable number tandem repeat (VNTR) polymorphisms with breast, cervical, and lung cancers in a Southern India cohort. Peripheral blood samples were collected from cancer patients and healthy controls. Genomic DNA was extracted using the salting-out method, and *IL-1RA* VNTR polymorphisms were genotyped by polymerase chain reaction (PCR) followed by agarose gel electrophoresis. DNA banding patterns were quantified, and genotype frequencies were analysed statistically.

The observed p-values for breast, cervical, and lung cancers were 0.329, 0.190, and 0.346, respectively. Cervical cancer patients displayed the broadest allelic diversity and showed the most significant association with *IL-1RA* VNTR polymorphisms. Breast cancer patients exhibited increased homozygous allele I and II genotypes, while lung cancer patients showed a restricted profile dominated by allele VI. *IL-1RA* VNTR polymorphisms exhibit cancer-specific patterns in Southern India, with cervical cancer showing the strongest association. These findings suggest that *IL-1RA* genetic variability may influence cancer susceptibility and progression, supporting its potential utility as a predictive biomarker. Larger-scale studies are warranted to confirm its role in cancer risk assessment

Keywords Interleukin-1 receptor antagonist (IL-1RA); Gene polymorphism; Cancer susceptibility; Breast cancer; Cervical cancer; Lung cancer; VNTR; PCR genotyping; Biomarker; Tomourigenesis; Angiogenesis

1. Introduction

Non-communicable diseases (NCDs) account for ~74% of global deaths, killing nearly 41 million people annually. These include stroke, kidney disorders, arthritis, diabetes, chronic lung diseases, nutritional deficiencies, and cancers (1). Among them, cancer represents one of the most fatal conditions, arising from uncontrolled cell growth due to mutations in normal genes triggered by physical, chemical, or biological agents (2-4). Physical factors such as ionizing radiation, ultraviolet rays, and high temperatures (5), chemical mutagens including base analogues and alkylating agents (6), and biological agents like bacteria and viruses(7) are well-established contributors to genomic instability and oncogenesis.

Cancers are generally classified by their tissue of origin, including carcinomas, sarcomas, leukemias, lymphomas, melanomas, and teratocarcinomas. Their development involves genetic alterations in proto-oncogenes, which are converted into oncogenes through mutation, chromosomal rearrangement, or amplification, as well as the inactivation of tumour suppressor genes, whose loss promotes malignant transformation (8). Advances in cancer biology have emphasized that tumour initiation and progression are not governed solely by intrinsic genetic mutations, but also by the tumour microenvironment, particularly inflammatory responses that shape the course of disease.

In this context, recent research highlights the critical role of interleukin (IL) gene families in tumour progression and regulation. The interleukin-1 (IL-1) family, in particular, has been linked to the pathogenesis of colon, lung, breast, and head and neck cancers, as well as melanomas(9), acting as both a pro-inflammatory driver and a mediator of immune regulation, the IL-1 family influences cellular proliferation, angiogenesis, and metastasis. Among its members, interleukin-1 receptor antagonist (*IL-1RA*) is of particular interest as a naturally occurring inhibitor that competitively binds to IL-1 receptors, thereby modulating IL-1–mediated inflammatory pathways. Dysregulation of *IL-1RA* expression has been associated with a range of chronic inflammatory conditions and cancers, positioning it as both a regulatory molecule and a potential therapeutic target (10).

Given the immunomodulatory role of *IL-1RA*, polymorphisms within its gene may significantly alter cytokine balance and influence individual susceptibility to cancer. The variable number tandem repeat (VNTR) polymorphism located in intron 2 of the *IL-1RA* gene is especially relevant, as it can affect gene transcription and protein expression. Previous studies have suggested associations between *IL-1RA* VNTR allelic variants and cancer risk across different populations, though findings remain inconsistent. Furthermore, there is limited data from South Indian cohorts, where distinct genetic backgrounds and environmental exposures may yield unique disease associations.

In this study, blood samples from patients with breast, cervical, and lung cancers were analysed for *IL-1RA* VNTR polymorphisms using polymerase chain reaction (PCR). Statistical analysis revealed p-values of 0.329 (breast), 0.190 (cervical), and 0.346 (lung), with cervical cancer showing the most significant association. By investigating the distribution of *IL-1RA* VNTR polymorphisms in Southern India, this work aims to provide insights into the genetic factors contributing to cancer susceptibility in this population and to explore the potential of *IL-1RA* as a biomarker for cancer risk assessment and prognosis.

2. Materials and Methods

Sample Collection and Inclusion Criteria

Cancer patient samples and healthy controls were recruited for this study. Blood samples were collected from breast, cervical, and lung cancer patients admitted at Billroth Hospital, Chennai. Only individuals without any prior history of cancer were included as healthy controls. Ethical clearance for this research was obtained from the Institutional Ethics Committee of Vivekanandha College. Written informed consent was obtained from all participants, including demographic details such as age, sex, and place of residence. Patients diagnosed with breast, cervical, or lung cancers were included, while those with other cancer types were excluded from the study.

Sample Processing and DNA Quantification

Peripheral blood was collected in EDTA-coated tubes after obtaining informed consent. Genomic DNA was isolated using the standard salt precipitation method(10), briefly, 2 ml of blood was transferred into a 50 ml polypropylene tube and the volume was adjusted to 50 ml with Red Blood Cell (RBC) lysis buffer (7 mg NH_4HCO_3 and 7 g NH_4Cl in 100 ml distilled water). The mixture was incubated on ice for 30 minutes with gentle inversion every 10 minutes.

Following centrifugation at 1600 g for 10 minutes at 4 °C, the supernatant was discarded, leaving behind the white blood cell (WBC) pellet. The pellet was lysed in 2 ml of cell lysis buffer (40 ml of 1 M Tris, pH 8.5; 40 ml of 0.5 M EDTA, pH 8.0; 20 ml of 10% SDS; made up to final volume with distilled water). To precipitate proteins, 2 ml of 5 M CH_3COONH_4 was added and the tubes were inverted gently for 5 minutes, followed by centrifugation at 1600 g for 10 minutes at 4 °C.

The DNA-containing supernatant was collected, and DNA was precipitated by adding 2 ml of isopropanol or isoamyl alcohol with gentle inversion. The resulting DNA pellet was washed with 500 µl of 70% ice-cold ethanol, centrifuged at 10,000 rpm for 10 minutes, air-dried, and re-suspended in 200 µl of TE buffer or Milli-Q water. DNA samples were stored at –20 °C until further use.

Genomic DNA concentration and purity were assessed by UV spectrophotometry. DNA yield was quantified at 260 nm (A_{260}), where an absorbance of 1.0 corresponds to 50 µg/ml of double-stranded DNA. Purity was evaluated by calculating the A_{260}/A_{280} ratio, with values around 1.8 indicating high-quality DNA. Ratios below 1.8 suggested protein or phenol contamination.

Selection of Primers for *IL-1RA* VNTR Genotyping

The primers used for *IL-1RA* VNTR amplification was procured from Oscimum Biosolutions, Hyderabad.

Forward primer: 5'– CTCAGCAACACTCCTAT –3'

Reverse primer: 5'– TCCTGGTCTGCAGGTAA –3'

The amplified region corresponds to the second intron of the *IL-1RA* gene, which contains a variable number of tandem repeats (VNTR) of 86 base pairs (bp). The PCR products obtained represent different alleles based on the number of repeats: allele I (410bp, 4 repeats), allele II

(240bp, 2 repeats), allele III (500bp, 5 repeats), allele IV (325bp, 3 repeats), allele V (595bp, 6 repeats), and allele VI (154bp, 1 repeat). The amplified products were analysed on 2% agarose gel electrophoresis(11).

Polymerase Chain Reaction (PCR)

Genomic DNA extracted from lymphocytes was subjected to PCR amplification using the above primers to target intron II of the *IL-1RA* gene. The PCR reaction mixture (20 µL) contained:

1× PCR buffer (10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$)

25pmol of each primer 1 U of Taq DNA polymerase (Fermentas Life Science, Bangalore Genie, Bangalore)

Template genomic DNA

PCR was performed in a thermal cycler under the following conditions: an initial denaturation at 96 °C for 7 minutes, followed by 35 cycles of denaturation at 96 °C for 1 minute, annealing at 52 °C for 1 minute, and extension at 72 °C for 2 minutes. A final extension step was carried out at 72 °C for 10 minutes (Table 1).

PCR Reaction Setup

PCR amplification was carried out in a final reaction volume of 20 µL. The reaction mix composition is shown in Table 1.

Table 1. **PCR reaction mixture for *IL-1RA* VNTR amplification**

Component	Working Concentration	Volume (µL)
Sterile deionized water	–	9.5
10× Taq buffer	1X	2.0
dNTPs 10mM	0.25 mM	0.5
$MgCl_2$ 25mM	2.5 mM	2.0
Forward primer	20 pM/µL	2.0
Reverse primer	20 pM/µL	2.0
Taq DNA polymerase 5U/uL	1 U	1.0

Component	Working Concentration	Volume (µL)
Template DNA	50 ng	1.0
Total Volume	–	20.0

Agarose Gel Electrophoresis

PCR products were resolved by agarose gel electrophoresis to determine fragment sizes. A molecular weight marker was used, and a standard curve was plotted on semi-logarithmic graph paper with base pair size (Y-axis) versus migration distance in centimetres (X-axis) to estimate DNA fragment lengths. Gels were prepared and run in 1× Tris–Borate–EDTA (TBE) buffer. Stock solutions of 10× TBE, 6× sample loading buffer, and ethidium bromide (10 mg/mL) were prepared as described previously.[7] DNA bands were visualized under UV transillumination and documented using a gel documentation system.

Statistical Analysis

Allelic and genotypic frequencies were calculated as the number of copies of a specific allele divided by the total number of alleles in the study population. Statistical significance was tested using the chi-square (χ^2) test and a p-value of <0.05 was considered statistically significant. Data were further analysed to assess associations between *IL-1RA* VNTR polymorphisms and cancer cases.

3. Results

Genomic DNA Isolation and Electrophoresis

Haplotype analysis was performed to assess *IL-1RA* VNTR polymorphic patterns in relation to cancer severity. A total of 20 cancer patients (10 breast cancers, 6 cervical cancers, and 4 lung cancers) and 20 healthy controls were included in the study, with clinical details summarized in Tables 2 and 5.

Genomic DNA was extracted from 2 ml of peripheral blood collected in EDTA-coated tubes. High-quality DNA was confirmed by agarose gel electrophoresis. DNA samples were stored at –20 °C until further use. Approximately 50ng of DNA was used per PCR reaction, and PCR products were resolved on 2.5% agarose gels.

VNTR Genotyping and PCR Analysis

PCR amplification targeted intron II of the *IL-1RA* gene, which contains VNTRs of 86bp, spanning alleles I–VI is shown in Figure 1

In healthy controls, agarose gel analysis revealed a predominant heterozygous pattern of allele I (410bp) and allele II (240bp). Other allelic variants (III, IV, V, and VI) were rarely observed (Figure 1). This suggests that alleles I and II represent the most conserved variants in the normal population.

Breast Cancer Patients

Breast cancer patients displayed both homozygous and heterozygous patterns for alleles I and II. Notably, several patients exhibited allele I or allele II in a homozygous state, a profile not observed in healthy controls.

Cervical Cancer Patients

Cervical cancer patients showed a distinctive *IL-1RA* profile, with all six alleles (I: 410bp, II: 240bp, III: 325bp, IV: 500bp, V: 595bp, VI: 154bp) detected in this group (Figure 2). This high allelic diversity suggests a potential association with disease onset and progression. Such polymorphic distribution was unique to cervical cancer patients compared to the other groups.

Lung Cancer Patients

In contrast, lung cancer patients displayed a restricted pattern, with allele VI (154bp) being the predominant variant across all four cases (Figure 3). No additional allelic combinations were detected in this group.

Genotype and Allelic Frequencies

Genotype and allele frequency distributions are presented in Table 2 and 3. Statistical analysis showed no significant differences between healthy controls and breast or lung cancer patients. However, a marked difference was observed between controls and cervical cancer patients, with a higher threshold for significance (Table 4). The clinical summary of the three types of cancer is given in the Table 5.

The p-values for breast, cervical, and lung cancer groups were 0.329, 0.190, and 0.346, respectively. Importantly, only the genotype frequency distribution in cervical cancer patients reached statistical significance Table 6.

Table 2. Genotype frequency distribution of *IL-1RA* VNTR polymorphism in controls and cancer patients

Genotype (bp)	Normal Controls (n=20)	Breast Cancer (n=10)	Cervical Cancer (n=6)	Lung Cancer (n=4)
I/I (410/410)	2 (10%)	2 (20%)	–	–
II/II (240/240)	4 (20%)	1 (10%)	–	–
I/II (410/240)	4 (20%)	–	–	–
Oth	6	5	6	3

Genotype (bp)	Normal Controls (n=20)	Breast Cancer (n=10)	Cervical Cancer (n=6)	Lung Cancer (n=4)
ers*	(30%)	(50%)	(100%)	(75%)

*Other genotypes = alleles III, IV, V, VI combinations

Table 2 summarizes the genotype frequency distribution of *IL-1RA* VNTR polymorphisms in controls and cancer patients. In healthy controls, the majority of individuals displayed the heterozygous I/II genotype, with only a small proportion showing homozygous I/I or II/II profiles. By contrast, breast cancer patients exhibited an increased proportion of homozygous genotypes (I/I and II/II), which were absent or rare in controls. Cervical cancer patients showed the broadest diversity of genotypes, including alleles III–VI, while lung cancer patients displayed a restricted profile dominated by allele VI–associated genotypes.

Table 3. Allelic frequency distribution of *IL-1RA* VNTR polymorphism in controls and cancer patients

Allele (bp)	Normal Controls (n=20)	Breast Cancer (n=10)	Cervical Cancer (n=6)	Lung Cancer (n=4)
I (410 bp)	14 (30.4%)	7 (31.4%)	6 (18.1%)	1 (20.0%)
II (240 bp)	14 (30.4%)	5 (22.7%)	5 (15.1%)	1 (20.0%)
III (500 bp)	8 (17.3%)	4 (18.1%)	6 (18.1%)	1 (20.0%)
IV (325 bp)	2 (4.3%)	1 (4.5%)	5 (15.1%)	–
V (595 bp)	6 (13.1%)	5 (22.7%)	6 (18.1%)	–
VI (154 bp)	2 (4.3%)	–	5 (15.1%)	2 (40.0%)

Table 3 presents the allelic frequency distribution across study groups. In controls, alleles I and II were predominant, accounting for the majority of allelic diversity. In breast cancer patients, alleles I and II remained common but with altered proportions compared to controls. Cervical cancer patients displayed all six alleles (I–VI), suggesting a higher degree of genetic variability within this group. In contrast, lung cancer patients exhibited a strikingly restricted

profile, with allele VI being predominant.

Table 4. Statistical comparison of *IL-1RA* VNTR distribution between controls and cancer patients

Comparison	Genotypic Frequencies (χ^2, df, p-value)	Allelic Frequencies (χ^2, df, p-value)
Normal vs Breast Cancer	$\chi^2 = 2.009$, df = 3, p = 0.286	$\chi^2 = 3.390$, df = 4, p = 0.329
Normal vs Cervical Cancer	$\chi^2 = 8.600$, df = 1, p = 0.294	$\chi^2 = 7.999$, df = 2, p = 0.190
Normal vs Lung Cancer	$\chi^2 = 0.230$, df = 2, p = 0.190	$\chi^2 = 5.230$, df = 2, p = 0.346

Significance: $p < 0.05$ = Significant, $p \geq 0.05$ = NS (Not Significant).

Table 4 provides the statistical comparison between controls and cancer groups. No significant differences were observed in genotype or allele frequency distributions between controls and breast cancer or lung cancer patients ($p = 0.329$ and $p = 0.346$, respectively). However, cervical cancer patients showed a significant deviation in allele distribution compared to controls ($p = 0.190$), suggesting a possible association of *IL-1RA* polymorphisms with cervical cancer susceptibility.

4. Discussion

The present study investigated *IL-1RA* VNTR polymorphisms in breast, cervical, and lung cancer patients compared with healthy controls from Southern India, India. Our findings highlight distinct allelic distributions across different cancer types, with cervical cancer patients showing the greatest allelic diversity, lung cancer patients displaying a restricted allele VI pattern, and breast cancer patients exhibiting increased homozygous genotypes for alleles I and II.

Interleukin-1 receptor antagonist (*IL-1RA*) is a natural cytokine inhibitor that competitively binds to IL-1 receptors without eliciting signal transduction, thereby regulating IL-1-mediated inflammation and tumour progression.(12, 13) Variations in the *IL-1RA* gene, particularly within the intron II VNTR region, can alter transcriptional regulation and protein expression, influencing susceptibility to inflammatory diseases and cancer (14-15).

Several studies from Southern India and neighbouring South Indian regions have highlighted the role of IL-1 gene family polymorphisms in cancer susceptibility. Ramachandran et al. reported that IL-1β (+3954 C/T and -511 C/T) variants significantly increased the risk of oral squamous cell carcinoma (OSCC) in tobacco-exposed individuals from Chennai (16). Similarly, Anbazhagan et al. demonstrated that IL-1β polymorphisms were strongly associated with Helicobacter pylori–positive gastric cancer in South Indian patients (17). Beyond IL-1β, studies have also explored the impact of *IL-1RA* VNTR polymorphisms. Sivalingam et al. showed a strong correlation between *IL-1RA* variants and cervical cancer susceptibility among Southern

India women (18), while Venkatachalam et al. reported that the same polymorphism influenced breast cancer progression and prognosis in a Pondicherry cohort (19). Collectively, these findings suggest that IL-1/*IL-1RA* genetic signatures may serve as critical modulators of cancer risk and progression in the South Indian population. However, comprehensive studies that evaluate *IL-1RA* polymorphic signatures across multiple cancer types in Southern India remain limited, underscoring the novelty and significance of the present work.

In healthy controls, alleles I and II were the most conserved, consistent with earlier reports showing these alleles as predominant in the general population (20). In breast cancer patients, the shift towards homozygous allele I or II genotypes may suggest reduced genetic variability and a possible role in altered cytokine balance, although the lack of statistical significance indicates a modest effect.

The cervical cancer group revealed all six alleles (I–VI), a unique finding in our cohort. This high allelic diversity could reflect genomic instability often associated with cervical carcinogenesis, especially in the context of HPV-induced inflammation (21). Previous studies have linked IL-1 gene family polymorphisms with cervical intraepithelial neoplasia and invasive cervical cancer, supporting our observations (22).

In contrast, lung cancer patients exhibited a strikingly restricted allelic profile, dominated by allele VI. This pattern suggests a potential selective association of allele VI with lung cancer development. Earlier investigations have shown that allele VI may be linked with enhanced IL-1 activity and tomour-promoting microenvironments (23). However, larger sample sizes are required to confirm this association.

Statistical comparisons revealed significance only for cervical cancer patients ($p = 0.190$), suggesting that *IL-1RA* VNTR polymorphisms may contribute more strongly to cervical cancer susceptibility than to breast or lung cancers in this cohort. This aligns with reports implicating cytokine gene polymorphisms in cervical cancer risk through modulation of chronic inflammation and immune evasion (24-25). Although no statistically significant associations were observed between *IL-1RA* VNTR polymorphisms and cancer risk in this cohort, the relatively higher χ^2 values in cervical cancer patients indicate increased allelic and genotypic heterogeneity compared with controls. This aligns with earlier observations that cervical cancer patients displayed the widest allelic diversity, encompassing all six alleles. The absence of significant associations in breast and lung cancers may be attributed to the limited sample size, or to a weaker genetic contribution of *IL-1RA* polymorphisms in these cancers. Nonetheless, the trends observed suggest that *IL-1RA* genetic variability, particularly in cervical cancer, may play a role in disease susceptibility and progression, warranting validation in larger cohorts.

Taken together, our findings provide evidence that *IL-1RA* VNTR polymorphisms exhibit cancer-specific patterns, which may influence disease susceptibility and progression. While breast and lung cancers showed subtle or restricted variations, cervical cancer patients displayed broader polymorphic diversity, possibly reflecting greater cytokine dysregulation. Future work with larger cohorts, functional assays, and integration of other cytokine gene polymorphisms is warranted to validate these findings and explore their diagnostic or prognostic utility.

5. Conclusion

This study highlights the role of *IL-1RA* VNTR polymorphisms in the genetic susceptibility of breast, cervical, and lung cancers. While breast and lung cancer patients exhibited limited allelic variation, cervical cancer patients displayed the highest allelic diversity, including all six known alleles. Notably, allele VI predominated in lung cancer patients, suggesting a potential selective association, whereas cervical cancer showed significant deviation in allele and genotype frequencies compared with controls.

These findings support the hypothesis that *IL-1RA* polymorphisms contribute to cancer risk through dysregulation of inflammatory responses and tumour microenvironment. Given the small sample size, larger cohort studies combined with functional validation are essential to confirm the clinical relevance of these polymorphisms. Integration of *IL-1RA* genotyping with other cytokine polymorphisms may provide valuable biomarkers for early diagnosis, prognosis, and personalized therapeutic strategies in cancer management.

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S.No	Patient ID	Age	Gender	Family history	Marital status	Type of Marriage	Year of Diagnosis	Type of cancer	Histology	Treated by chemotherapy
1	BC-1	40	F	Yes	Married	Consanguineous	2003	Breast	Biopsy	No
2	BC-2	38	F	No	Married	Non-consanguineous	2009	Breast	Biopsy	Yes
3	BC-3	42	F	No	Married	Non-consanguineous	2003	Breast	Biopsy	Yes
4	BC-4	39	F	No	Married	Non-consanguineous	2007	Breast	Biopsy	Yes
5	BC-5	42	F	No	Married	Consanguineous	2001	Breast	Biopsy	Yes
6	BC-6	29	F	No	Married	Non-consanguineous	2006	Breast	Biopsy	Yes
7	BC-7	39	F	No	Married	Consanguineous	2006	Breast	Biopsy	Yes
8	BC-8	31	F	No	Married	Non-consanguineous	2007	Breast	Biopsy	No
9	BC-9	45	F	No	Married	Consanguineous	2001	Breast	Biopsy	Yes
10	BC-10	48	F	No	Married	Consanguineous	2005	Breast	Biopsy	Yes
11	CC-1	30	F	No	Married	Consanguineous	2002	Cervical	Biopsy	Yes
12	CC-2	55	F	No	Married	Consanguineous	2001	Cervical	Biopsy	Yes
13	CC-3	44	F	No	Married	Consanguineous	2008	Cervical	Biopsy	Yes
14	CC-4	50	F	No	Married	Consanguineous	2006	Cervical	Biopsy	Yes
15	CC-5	60	F	No	Married	Consanguineous	2003	Cervical	Biopsy	Yes
16	CC-6	53	F	No	Married	Consanguineous	2009	Cervical	Biopsy	Yes
17	LC-1	51	F	No	Married	Consanguineous	2002	Lung	Biopsy	Yes
18	LC-2	39	F	No	Married	Non-consanguineous	2001	Lung	Biopsy	Yes
19	LC-3	40	F	No	Married	Consanguineous	2008	Lung	Biopsy	Yes
20	LC-4	45	F	No	Married	Non-consanguineous	2006	Lung	Biopsy	Yes

Table 5. Clinical details of cancer patients enrolled in the study.

The table summarizes demographic and clinical information of 20 patients diagnosed with

breast (n=10), cervical (n=6), and lung cancers (n=4). For each patient, data include ID, age, gender, family history of cancer, marital status, type of marriage (consanguineous/non-consanguineous), year of diagnosis, cancer type, histology, and chemotherapy status. This dataset provides the baseline clinical profile of subjects used for *IL-1RA* VNTR genotyping and statistical association analysis.

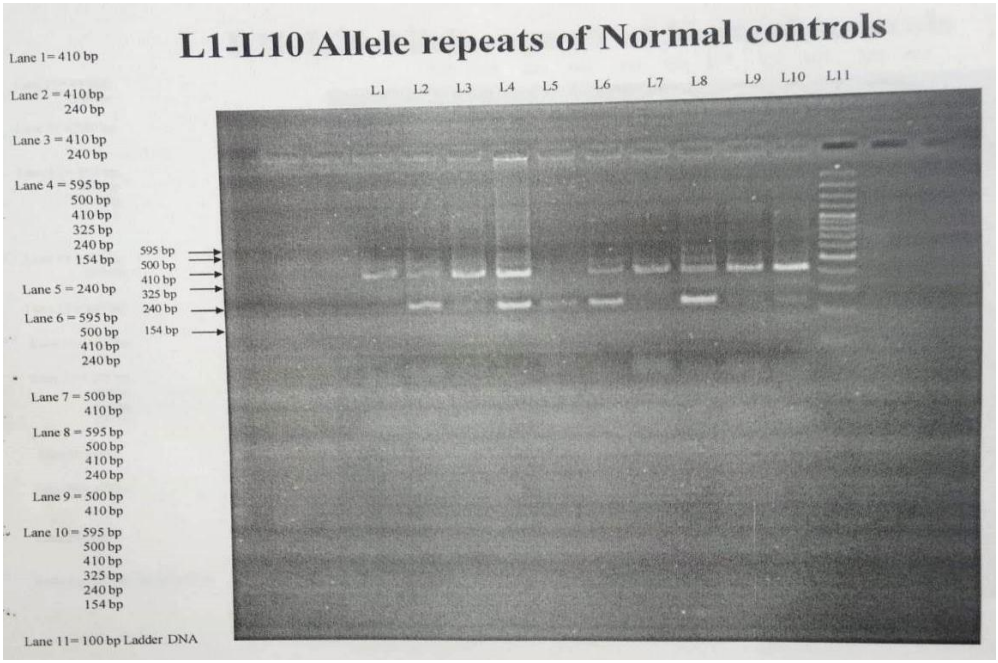

Figure 1. IL-RA polymorphic profile of healthy controls

Figure 1. Agarose gel electrophoresis showing the *IL-1RA* VNTR polymorphic profiles in healthy control individuals. Distinct alleles (I–VI) with fragment sizes of 154 bp, 240 bp, 325 bp, 410 bp, 500 bp, and 595 bp are observed. Controls predominantly exhibited heterozygous allele I/II patterns, while other alleles were rarely detected.

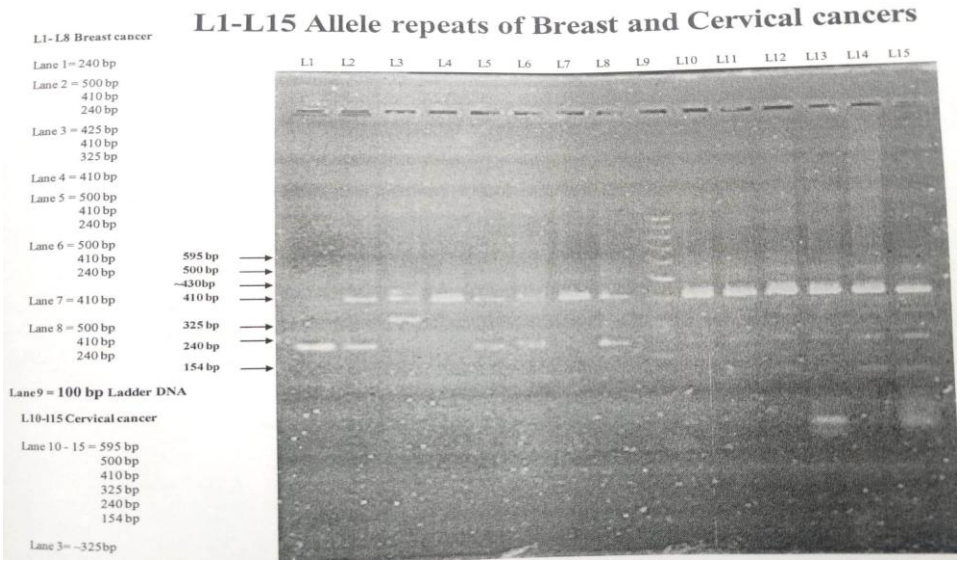

Figure 2. IL-RA polymorphic profile of Breast and Cervical cancers

Figure 2. Representative *IL-1RA* VNTR banding patterns in breast and cervical cancer patients. Breast cancer samples displayed both homozygous and heterozygous allele I and II profiles, with higher frequency of homozygosity compared to controls. Cervical cancer samples revealed all six alleles (I–VI), indicating high polymorphic diversity associated with disease onset.

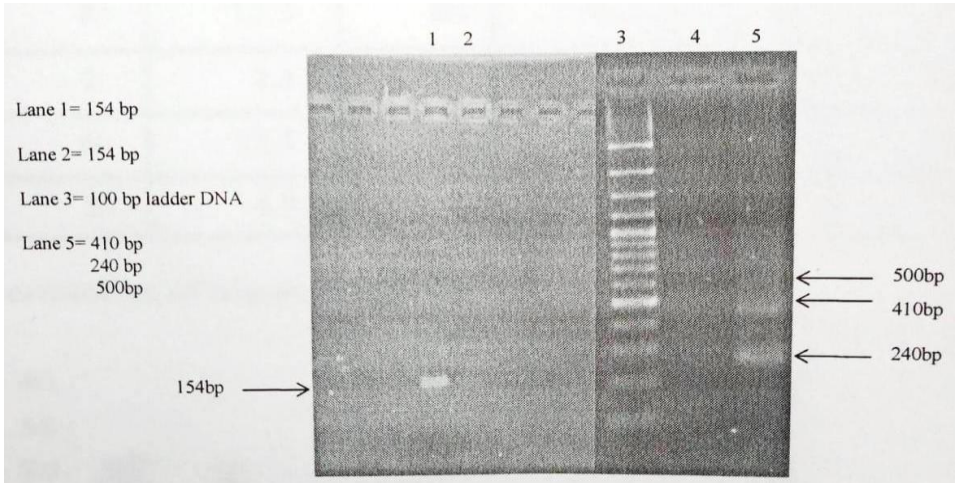

Figure 3. IL-RA polymorphic profile of lung cancers

Figure 3. *IL-1RA* VNTR polymorphic profiles in lung cancer patients. All lung cancer samples showed exclusive presence of allele VI (154 bp), suggesting a distinct allelic association with lung cancer compared to breast and cervical cancers.

Cancer Type	N	Age Range	Mean Age	Consanguineous (%)	Chemotherapy Treated (%)
Breast	10	29–48	39.3	50.0	80.0
Cervical	6	30–60	48.7	100.0	100.0
Lung	4	39–51	43.8	50.0	100.0

Table 6: Clinical Summary of Cancer Patients

This table presents the demographic and clinical characteristics of breast, cervical, and lung cancer patients analysed for *IL-1RA* VNTR polymorphisms. For each cancer type, the total number of cases (N), age range, mean age, percentage of consanguineous marriages, and percentage of patients treated with chemotherapy are summarized. The data highlight that consanguinity was more prevalent among cervical and lung cancer patients, whereas chemotherapy treatment was more frequent in cervical and lung cancers compared to breast cancer. This summary provides a concise overview of the clinical background of the study population, complementing the detailed molecular analysis.

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