Association of human papillomavirus and breast tumours

Health & Medicine

Abstract:

Cancer arises due to abnormal changes or mutations, in the genes responsible for regulating the growth of cells. Human papillomavirus (HPV) is considered the aetiological agent for many cancers including cervical cancer. HPV causes disruption and loss of some of the viral genes such as L1 and L2 genes and also increases the expression of the early genes. Several studies have addressed a relationship with HPV and breast cancer, as different HPVs have been identified. Most of the studies were successful in finding evidence in correlation of HPV 6, 11, 16 and 18 in invasive ductal breast carcinoma by using different techniques including DNA extraction and PCR, however other studies achieved low positivity or negative result. The aim of this study was to find out the association of HPV and Breast cancer. DNA was successfully extracted from archived breast tissue samples using DNA extraction method. This DNA sample could be amplified by using PCR to find HPV genome specifically targeting E1 gene. This is an ongoing work by the supervisors of the project to try and detect HPV genome in breast cancer, if successful a vaccine could be developed against various strains of HPVs worldwide and it could save many lives.

Keywords: Human papillomavirus, DNA Extraction, Breast cancer, Vaccine, PCR,

1. Introduction:

1. 1. Breast cancer:

Breast cancer is a malignant tumour that originates in the breast tissue, mainly from the inner lining of milk ducts or the lobules that supply milk to

the ducts, cancers that initiates from ducts are called ductal carcinomas and those originating from lobules are called as lobular carcinomas. Cancer occurs due to abnormal changes or mutations, in the genes responsible for regulating the growth of cells (Sariego, 2010). The change in the genetic information causes a cell to no longer carry out its function properly (Almeida & Barry, 2010). The following figures show the two types of cancers Benign and Malignant.

(Almeida; Shela, 2010)Figure: 2malignant tumours

Figure 1 and 2: above shows benign vs. malignant cancers. (a) A benign tumour is a mass of cells that remains within the tissue in which it originally developed. (b) The invasion of cancer cells into surrounding tissues is the hallmark of a malignant tumour. Malignant cells may break free from the tumour and travel to other locations in the body through the process of metastasis (Almeida & Barry, 2010).

1. 2. Epidemiology:

Breast cancer is one of the mainhealthproblems worldwide (Bao, 2011) and which resulted http://en. wikipedia. org/wiki/Breast_cancer – cite_note-WHO_WCR-2 458, 503 deaths in 2008 worldwide out of which 13. 7% are of cancer deaths in women and it is about 100 times more common in women than in men (Veto, et al., 2009).

The table below shows how females are susceptible to breast cancer at different ages for example there is 1 in 8 risk of developing breast cancer in females in the U. K in lifetime.

Table 1: Shows estimated risk of developing breast cancer by age, females, UK, 2008

UK, 2008

Adopted from: www. cancerresearch. uk

http://info. cancerresearchuk. org/cancerstats/types/breast/riskfactors/

Date accessed: 20/01/11

The table 2 below shows that more deaths happens in females due to breast cancer than males as it can be seen from the table only 69 males died in 2008 in compare to 12, 047 females.

Table 2: Shows the number of deaths and mortality rates in the UK in 2008.

Adopted from: www. cancerresearch. uk

http://info. cancerresearchuk. org/cancerstats/types/breast/mortality/#age

Date accessed: 20/01/11

The figure below shows the incidence and mortality rates from female breast cancer in EU countries. As it can be seen from the table Belgium has the highest rates of incidence in female breast cancer.

Figure 1 above is a graph of incidence and mortality rates in EU.

Adopted from: www. cancerresearch. uk

http://info. cancerresearchuk. org/cancerstats/types/breast/incidence/

Date accessed: 20/01/11

1. 3. Breast cancer Pathophysiology:

1. 3. 1Aetiology:

Some of the suspected aetiological factors which influence the cases of breast cancer arefamilyhistory, obesity, age, oral contraceptives and alcohol.

Family history: A woman who has a family member with breast cancer increases double the risk of getting breast cancer in compare to a woman with no family history (Lancet, 2001).

Obesity: obesity increases the risk of postmenopausal breast cancer by up to 30%, since levels of hormones rises with excess body fat such as oestrogen and insulin these are the common features of cancers.

Age: older women are at higher risk. Particularly women aged 50-69 are most at risk, predominantly those with a late menopause.

Oral contraceptives: increases the risk by approximately a quarter but since people who uses are commonly younger women, therefore the risk is fairly low.

Alcohol: drinking alcohol as less as one alcoholic drink each day increases the risk of breast cancer by around 12%.

(Cancer Research U. K, 2008)

Some other factors include:

Lesions to DNA such as genetic mutations. There is link between mutations that can lead to breast cancer and oestrogen exposure, found out by carrying out experiments.

Another factor is when a body fails to carry out immune surveillance; it is a theory in which the immune system gets rid of malignant cells throughout one’s life.

Other factor is inherited defects in DNA repair genes, such as ” BRCA1”, ” BRCA2” and ” TP53” (Adams, et al., 2011).

Figure 2 above shows the percentage of different genes with associated risk.

Figure adopted from: Wooster and Webber, (2003)

Date accessed: 12/04/11

Moreover according to many authors there is a potential link between the HPV and breast cancer.

1. 4. Human papillomavirus and Cancer:

HPV genome is normally found in the cytoplasm of infected tissues however, the DNA of HPV types that cause cancer are integrated into the host genome. HPV causes disruption and loss of some of the viral genes for example (L1 and L2 genes) and also increases the expression of the early genes (Wang, 2007; Mera, 1997).

Oncoproteins E5 interacts with MHC I and prevents its transport to the cell surface therefore infected cells escapes the immune system consequently allowing the virus to establish persistent infections and thus progressing to cancer.

E6 targets p53 for degradation and therefore prevents apoptosis of abnormal cells, whereas E7 inactivates Rb (retinoblastoma) function, which results in abnormal cell proliferation and disturbs the normal cell cycle regulation (Wang, 2007; WHO, 2006; Mera, 1997). P53 and Rb are tumour suppressor genes which stop tumours from developing (Mera, 1997). Incorporation of virus into host cell increases and sustains the growth of both virus and the host cell, thus resulting in the alteration of infected host cells into malignant cells (Mera, 1997; Wang, 2007) and ultimately invasive cancer.

Figure 3 above shows different genes in HPV.

Adopted from: Symptoms of HPV 2010

symptomsofhpv. net/113/hpv-16/

Date accessed: 07/04/11

Table 9 below shows the function of different genes within the HPV virus:

Gene/RegionFunction
E1/E2Code for proteins which control the function of E6 and E7 genes.
E4Function largely unknown but may control virus release from cell.
E5Codes for a hydrophobic protein which enhances immortalisation of the cell.
E6Codes for proteins which inhibit negative regulators of the cell cycle . E6 products inhibit p53 which is a transcription factor for apoptosis (programmed cell death).
E7Codes for products whichbind to the retinoblastoma tumour

suppressorproteins thereby permitting the cell to progress through the cell cycle in the absence of normal mitogenic signals.

L1/L2Code for structural proteins and formation of complete virus particles.

LCRNecessary for normal virus replication and control of gene expression.

Adopted from: Eurocytology

http://www. eurocytology. eu/static/eurocytology/eng/cervical/LP1ContentMcontA1. html

Date accessed: 19/03/11

The HPV (human papillomavirus) is a member of the papillomaviridae family and has a double stranded circular DNA genome (Wang, 2007). These viruses are small in size with 8kbp-long DNA genome and have no envelope (WHO, 2006).

HPV genome contains early (E) and the late genes (L) which codes for early proteins (E1-E7), late proteins (L1 and L2) and a non coding long control region (LCR) (WHO, 2006; Mera, 1997; Govan, 2008).

1. 4. 1. High risk and low risk HPV types:

There are more than one hundred different HPV types that have been discovered (WHO, 2006) and these are divided into high risk and low risk types. HPV 16, 18, 31 and 45 are some high risk HPV types associated with most of the cancer, while HPV 6 and 11 are low risk non-oncogenic HPV types (Brown, et al., 2005; Govan, 2008).

Table3: the following table shows some high risk, low risk and potentially risk HPVs.

Classification	HPV types
High-risk	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59
Low-risk	82, 6, 11, 40, 42, 43, 44, 54, 61, 70
Potentially high –risk	26, 53

Source: Govan (2008)

HPV 6 and 11 are linked with up to 90% genital warts (Von Krogh, 2011), nevertheless after the examination of 55 genital wart samples from Slovenia, using polymerase chain reaction (PCR), the authors concluded that HPV 6 and 11 genotypes were detected in 96. 4% of genital warts patients (Potocnik, et al., 2007).

1. 5. Signs and Symptoms:

Changes that could arise due to a breast cancer are:

A change in the size or shape of a breast

A lump or thickening in an area of the breast

Dimpling of the skin

A change in the shape of the nipple, particularly if it turns in, sinks into the breast or becomes irregular in shape

A blood stained discharge from the nipple

(Dixon, 2005; Breast cancer, 2008).

Figure 3a: above shows the symptoms of breast cancer

Source: Healthbase (2008)

http://blog. healthbase. com/2008_09_01_archive. html

Accessed date: 11/04/2011

Normal anatomy of the breast:

Female breast anatomy

The structure of female breast is mainly made up of fat and connective tissue, but also contains milk ducts, lymph nodes, blood vessels and structures known as lobes and lobules (Rosen, 2009).

Figure 4 above shows normal anatomy of breast tissue.

The above figure adopted from: Mayoclinic (2009)

http://www. mayoclinic. com/health/breast-cancer-early-stage/BC00001

Date accessed: 8 April 2011.

Lobules and ducts

Every breast has 12 to 20 lobules that protrude from the nipple and holds small alveoli; the lobules are connected together by a network of thin ducts (Rosen, 2009).

Figure 5 above shows different parts in the female breast

The above figure adopted from: Mayoclinic (2009)

http://www. mayoclinic. com/health/breast-cancer-early-stage/BC00001

Date accessed: 8 April 2011.

Stromata

Spaces around the lobules and ducts are filled with fatty tissue, ligaments and connective tissue (stromata). The size of the breast is determined by the amount of fat it contains, the breast tissue is also sensitive to cyclic changes in hormone levels (Rosen, 2009).

Figure 6 above shows the position of stromata in female breast.

The figure adopted from: Mayoclinic (2009).

http://www. mayoclinic. com/health/breast-cancer-early-stage/BC00001

Date accessed: 8 April 2011.

Muscles

Breasts are muscle free tissues, muscles lie beneath the breasts separating them from the ribs (Rosen, 2009).

The above figure adopted from: Mayoclinic (2009)

http://www. mayoclinic. com/health/breast-cancer-early-stage/BC00001

Date accessed: 8 April 2011.

Arteries and capillaries

Blood supply all the essential nutrients and oxygen to the breast tissue through arteries, capillaries and small blood vessels (Rosen, 2009).

Figure 8 above shows the position of capillaries and arteries in and around the breast.

Figure adopted from: Mayoclinic, (2009)

http://www. mayoclinic. com/health/breast-cancer-early-stage/BC00001

Date accessed: 8 April 2011.

Lymph nodes and lymph ducts

The lymphatic system contains blood vessels, lymph ducts and lymph nodes that helps fight infection, lymph nodes are present behind the breastbone, under the armpit and in other parts of the body engulfs harmful substances that are in the lymphatic system and safely get rid of them (Rosen, 2009; Mayoclinic, 2009).

Figure 9 above shows the position of the lymph nodes and lymph ducts.

The above figure adopted from: Mayoclinic, 2009

http://www. mayoclinic. com/health/breast-cancer-early-stage/BC00001

Date accessed: 8 April 2011.

1. 4. Different types of Breast cancers:

There are different types of breast cancer for example ductal and lobular and it depends on the type of tissue that it is derived from.

Table 3. 1 below shows the list of different types of breast cancer:

DCIS – ductal carcinoma in situ

LCIS – lobular carcinoma in situ

Invasive ductal breast cancer

Invasive lobular breast cancer

Inflammatory breast cancer

Paget’s disease

Breast cancer in men

The following figures show some of the main types of the cancer that begins in different areas of the breast for example the ducts, the lobules, or in some cases, the tissue in between. These figures also show the different types of breast cancer, including non-invasive, invasive, metastatic and recurrent breast cancers.

a. Ductal Carcinoma in situ (DCIS)

Range of Ductal Carcinoma in situ (DCIS)

Figures: 10 and 11above show normal breast with non–invasive ductal carcinoma in situ (DCIS) in an enlarged cross–section of the duct.

Adopted from: Breast cancer (2008)

http://www. breastcancer. org/symptoms/types/dcis/diagnosis. jsp

Accessed date: 20/01/2011

Breast profileABCDEFG

DuctsLobulesDilated section of duct to hold milkNippleFatPectralis major muscleChest wall/ rib cage

EnlargementDuctal cancer cellsNormal lobular cellsBasement membraneLumen (centre of duct)

Table 4: shows the annotation of the above figures

(Trentham-Dietz, et al., 2011)

b. Lobular Carcinoma in situ (LCIS

Figure: 12above shows normal breast with lobular carcinoma in situ (LCIS) in an enlarged cross–section of the lobule.

Adopted from: Breast cancer (2008)

http://www. breastcancer. org/symptoms/types/ilc/tests/diagnosing. jsp

Accessed date: 20/01/2011

Table 5 shows the annotation of the figure 12.

Breast profileABCDEFG

DuctsLobulesDilated section of duct to hold milkNippleFatPectralis major muscleChest wall/ rib cage

EnlargementNormal Ductal cellsLobular cancer cellsBasement membrane

(Trentham-Dietz et al., 2011)

a. Invasive Ductal Carcinoma (IDC)

Figure 13above shows normal breast with invasive ductal carcinoma (IDC) in an enlarged cross–section of the duct.

Adopted from: Breast cancer (2008)

http://www. breastcancer. org/symptoms/types/ilc/tests/diagnosing. jsp

Accessed date: 20/01/2011

Breast profileABCDEFG
DuctsLobulesDilated section of duct to hold milkNippleFatPectralis major muscleChest wall/ rib cage
EnlargementNormal duct cellsductal cancer cells breaking through the basement membrane Basement membrane

Table 6 shows the annotation of the figure 13.

(Trentham-Dietz, et al., 2011)

c. Invasive Lobular Carcinoma (ILC)

The abovefigure 14shows normal breast with invasive lobular carcinoma (ILC) in an enlarged cross–section of the lobule.

Adopted from: Breast cancer (2008)

http://www. breastcancer. org/symptoms/types/ilc/tests/diagnosing. jsp

Accessed date: 20/01/2011

Table 7 shows the annotation of the figure 14.

Breast profileABCDEFG

DuctsLobulesDilated section of duct to hold milkNippleFatPectralis major muscleChest wall/ rib cage

EnlargementNormal cellsLobular cancer cells breaking through the basement membraneBasement membrane

(Trentham-Dietz, et al., 2011)

Following are some examples of non-invasive cell growths:

d. Non-Invasive Cell Growth Subtypes – Solid

Figure: 15showsAcancer cellsBbasement membrane

Adopted from: Breast cancer (2008)

Accessed date: 20/01/2011

e. Non-Invasive Cell Growth Subtypes – Cribriform

Figure: 16above shows(A)cancer cells (B)basement membrane (C)lumen (centre of duct)

Adopted from: Breast cancer (2008)

Accessed date: 20/01/2011

f. Non-Invasive Cell Growth Subtypes – Papillary

Figure: 17above shows(A)cancer cells (B)basement membrane (C)lumen (centre of duct)

Adopted from: Breast cancer (2008)

Accessed date: 20/01/2011

g. Non-Invasive Cell Growth Subtypes – Comedo

Figure: 18above shows(A)living cancer cells (B)dying cancer cells (C)cell debris (necrosis)

Adopted from: Breast cancer (2008)

Accessed date: 20/01/2011

h. Vascular and Lymphatic Invasion

Figure: 20above shows normal breast with cancer cells invading the lymph channels and blood vessels in the breast tissue

Adopted from: Breast cancer (2008)

Accessed date: 20/01/2011

Table 8 shows the annotation of the above figure

Breast profileABCDEF

Blood vesselsLymphatic channels

EnlargementNormal duct cellscancer cellsBasement membraneLymphatic channelBlood vesselBreast tissue

1. 4. Diagnostic tests:

Diagnosis of the breast cancer incorporates x-rays and screening tests and following are some of the importantdiagnostictests that can be carried out before and after symptoms of breast cancer.

Tests:

Mammogram:

A mammogram is the main screening test for asymptomatic patients who are over the age of 40 as well as for symptomatic adult patients (Bao, 2011). This test has a high sensitivity and specificity (Banks, 2004). If a mammogram does not find out an abnormality in patients with a clinically detected breast mass, additional imaging ultrasound and/or MRI should be carried out for further evaluation (Bao, 2011).

Outcome:

The results are indicative of malignancy include: an irregular speculated mass, clustered micro-calcifications, and linear branching calcifications (Banks, 2004; breast cancer, 2010).

The above figure 21 shows how mammography is carried out.

Figure 21 adopted from: Breast cancer (2010)

http://www. breastcancer. org/symptoms/testing/types/mammograms/

Accessed date: 02/04/2011

Breast Ultrasound:

Ultrasound sends high-frequency sound waves through the breast and changes them into images on a screen. The ultrasound technician places a sound-emitting probe on the breast to carry out the test and there is no radiation involved (Matsuzaki, et al., 2010).

Outcome:

The results are indicative of malignancy include: a hypo echoic mass, an irregular mass with internal calcifications, and enlarged auxiliary lymph nodes (breast cancer 2010; Moss, 1999).

The above figure 22 shows how ultrasound is carried out.

adopted from: Breast cancer (2010)

http://www. breastcancer. org/symptoms/testing/types/ultrasound. jsp

Accessed date: 02/04/2011

Breast MRI:

MRI uses magnets and radio to produce detailed cross sectional images of the inside of the body. MRI screens high-risk women (breast cancer, 2010).

The Sensitivity is 88% to 91%. Specificity is about 67% (Bluemke, 2004).

Outcome:

The results are indicative of malignancy include: a heterogeneously enhancing area and significant architectural distortion (Bluemke, 2004).

The above figure 23 shows how MRI is carried out.

adopted from: Breast cancer (2010)

http://www. breastcancer. org/symptoms/testing/types/mri/

Accessed date: 02/04/2011

Biopsy:

There are different types of biopsy techniques and among these techniques Fine needle aspiration (FNA) is the least invasive procedure and has high sensitivity and specificity (Dayal, et al., 2011). FNA is good for quick diagnosis of malignancy. Nonetheless, core biopsy is generally favoured, as it effectively differentiates between pre-invasive and invasive disease and is less chance getting inadequate sampling (Dayal, et al., 2011).

Outcome:

Invasive ductal carcinoma is responsible for almost 80% of all breast cancers, cords of tumour cells among associated glandular formation, which make varying degrees of fibrotic response. Whereas invasive lobular carcinoma, small tumour cells that invade past the basement membrane of the lobules and form an “ Indian file” between collagen bundles, usually appears as well-differentiated tumour cells that display tubule formation (Dayal, et al., 2011).

1. 5. Aims and Objectives:

The aim of this project was to evaluate the association of Human papillomavirus (HPV) and breast cancer, additionally to collect the studies that support the presence of HPV DNA in patients with breast lesions worldwide. The archived samples diagnosed with breast carcinoma, will be used to extract the DNA by DNA Extraction method which could be further used for amplifying this DNA using PCR to detect HPV genome. This will ascertain the role of this virus in the pathogenesis of breast cancer and will also help the scientist for further investigation of this virus on biology of cancer.

The following is the methodology of my project as how I carried out the experiment and extracted the DNA.

2. Methodology:

The methodology incorporates materials and method, health and safety, ethical issues and statistical analysis.

2. 1. Method and Materials:

The following table 9 shows the materials that have been used to extract the DNA.

MaterialsMeasurements

Universal tubes

20 ml, 5ml

Epindorf tube

1. 5 ml, 500ul

Gilson pipetts

2x 5- 50ul, 2x 0. 1 – 2. 5 ul, 2x 100 – 1000ul, 1x 20 – 200ul

Dry heat block (incubator)

Vortex

Waterbath (37c)

Centrifuge and microfuge

70% of alcohol to avoid

contamination and spray bottle

Ice box

10. Thermometer(to measure the temperature)

11. Spectrophotometer(OD reader)

12. Pipett tips

13. Tissue and Cell LysisSolution 600ul (60ml)

14. Proteinase K4ul (200ul)

15. RNase A2ul (400ul)

16. Protein Precipitation Reagent300ul (60ml)

17. Isopropanol1ml (2ml)

18. Ethanol70%

19. TE Buffer35ul (8ml)

2. 2. DNA purification method from tissue:

The following is the method used to extract the DNA from the archived

sample of breast tissue.

Lysis of Formalin-Fixed, Paraffin-Embedded (FFPE) Tissue

i. Placed 10-50 mg of 10- to 35-µm thick paraffin sections into an appropriate tube. If using a larger amount of tissue, adjust the reagent volumes accordingly.

ii. Diluted 4 µl of “ Proteinase K” into 600 µl of “ Tissue and Cell Lysis Solution” for each sample, and mixed.

iii. Added 600 µl of “ Tissue and Cell Lysis Solution” containing the “ Proteinase K” to the sample and mixed.

iv. Incubated at 65°C for 30 minutes; followed by a brief (10 seconds) vortex mix.

v. Cooled the samples to 37°C and added 2 µl of “ RNase A” to the sample; mixed thoroughly.

vi. Incubated at 37°C for 30 minutes.

vii. Placed the samples on ice for 3-5 minutes and then preceded with total DNA precipitation (below).

Precipitation of Total DNA

Added 300 µl of “ MPC Protein Precipitation Reagent” to 600 µl of lysed sample and vortex vigorously for 10 seconds.

ix. Pellet the debris by centrifugation at 4°C for 10 minutes at ? 10, 000 x g in a microcentrifuge. If the resultant pellet was clear, small, of loose, added an additional 25 µl of “ MPC Protein Precipitation Reagent”, mixed, and pellet the debris again.

x. Transferred the supernatant to a clean microcentrifuge tube and discarded the pellet.

xi. Added 500 µl of “ isopropanol” to the recovered supernatant. Inverted the tube 30-40 times.

xii. Pellet the DNA by centrifugation at 4°C for 10 minutes in a microcentrifuge.

Carefully poured off the “ isopropanol” without dislodging the DNA pellet.

Rinsed twice with 70% “ ethanol”, being careful to not dislodge the pellet.

Centrifuged briefly if the pellet was dislodged. Removed all of the residual ethanol with a pipet.

xv. Resuspended the DNA in 35 µl of “ TE Buffer”.

Source: Epicentre Biotechnologies

3. Result:

DNA is extracted by a DNA histological processing using PCR and DNA extraction techniques. These are techniques used to extract, amplify and copy small segments of DNA. It is fast and inexpensive because significant amounts of a sample of DNA are necessary for molecular and genetic analyses (Mendizabal et al., 2008).

3. 1. DNA Extraction:

DNA was extracted by using DNA extraction protocol written in the method section. In the DNA extraction different solutions were used for example Proteinase K enzyme is used to digest protein and to remove protein contamination from DNA and to get to the pure DNA (Ebeling, et al., 1974). Also different machines incubators, vortex and centrifuge were used to break down cell walls. Following the DNA extraction PCR is used to amplify the DNA to find HPV genome.

3. 2. PCR:

Using the PCR to amplify a segment of DNA firstly the sample is heated so that the DNA denatures or divides into two pieces of single-stranded DNA. After that an enzyme called “ Taq polymerase” synthesizes – builds – two new strands of DNA, using the original strands as templates. This process causes the duplication of the original DNA. Each of the molecules now carries one old and one new strand of DNA. After that each of these strands can be utilized to form two new copies, and the process continues in this manner. More than one billion exact copies of the original DNA segment is achieved by repeating the cycle of denaturing and synthesizing new DNA 35 or 40 times. This whole process of PCR is automated and can be done in just a few hours. A thermocycler machine directs this process and is programmed to change the temperature of the reaction every few minutes to cause DNA denaturing and synthesis.

Source: Bruce Fouke’s lab

Accessed date: 20/01/11.

There are many different types of PCR for example conventional PCR assays using consensus primers and highly sensitive Real-Time PCR (Hedau, et al., 2011).

Following are the result of the DNA extracted using a machine called nanoviewer. Many concentration of the DNA extracted are within the good range which is 1. 8 – 2. 0. This indicates that the samples have not been contaminated with protein.

The following table 10 shows the result of the DNA extracted:

Breast Tissue sample numberConcentration ng/ulResults

33101. 5 ng/ulA260/280 = 1. 990

847. 5 ng/ulA260/280 = 1. 939

7733 ng/ulA260/280 = 1. 886

5415. 9 ng/ulA260/280 = 1. 904

76105. 5 ng/ulA260/280 = 1. 835

2593. 0 ng/ulA260/280 = 1. 958

1229. 5 ng/ulA260/280 = 1. 735

1326. 0 ng/ulA260/280 = 1. 877

7143. 0 ng/ulA260/280 = 2. 014

4. Health and Safety:

The health and safety procedures were followed according to the requirement of the laboratory for this project and a copy of COSSH assessment was given to the laboratory technical staff and to the project supervisor

5. Ethical Issues:

Approval of UK'S ethical committee regarding the usage of the breast tissue samples has already been granted to the project supervisor and hence there is no need of further ethical approval for this project.

6. Literature search:

To understand the scope of the HPV and breast cancer very well 15 abstracts have been submitted at the beginning to the project supervisor that were conducted by many journals and research papers.

7. Statistical analysis:

This project is a laboratory based and therefore does not require any statistical analysis to be carried out.

8. Treatment:

Breast cancer is treated with surgery, radiotherapy, chemotherapy and drugs. There are many drugs that are used to either treat or reduce the risk of breast cancer and following are some example of these drugs:

8. 1. Drugs:

Table11 below shows the list of drugs used for breast cancer treatment

Herceptin (chemical name: Trastuzumab)

Tamofen (chemical name: Tamoxifen)

Arimidex (chemical name: anastrozole)

Aromasin (chemical name: exemestane)

Avastin (chemical name: bevacizumab)

Carboplatin (brand name: Paraplatin)

Cytoxan (chemical name: cyclophosphamide)

Daunorubicin (brand names: Cerubidine, DaunoXome)

Doxil (chemical name: doxorubicin)

Ellence (chemical name: epirubicin)

Thiotepa (brand name: Thioplex)

Trelstar (chemical name: triptorelin)

Tykerb (chemical name: lapatinib)

Vincristine (brand names: Oncovin, Vincasar PES, Vincrex)

Xeloda (chemical name: capecitabine)

Some of the drugs that are used are explained below.

Tamoxifen: is a drug that uses SERMs (selective oestrogen receptor modulator) that attaches to the oestrogen receptors in breast cells and blocks the effects of oestrogen (Lacroix, et al., 2010).

Uses: to treat men and both pre-menopausal and post-menopausal women, typically is used to:

shrink large, hormone-receptor-positive breast cancers before surgery

reduce breast cancer risk in undiagnosed women at higher-than-average risk of developing breast cancer

However Tamoxifen is very cost effective (Noah-Vanhoucke, et al., 2011)

Side effects:

irregular menstrual cycles

vaginal discharge or bleeding

depression

endometrial cancer

8. 2. Trastuzumab:

is a drug that uses HER2 (human epidermal receptor 2) inhibitors that works against HER2-positive breast cancers by blocking the ability of the cancer cells to receive chemical signals that tell the cells to grow.

Uses:

treat metastatic, HER2-positive breast cancer (Barok, et al., 2011)

shrink large, advanced-stage, HER2-positive cancers before surgery

Side effects:

diarrhea

anemia

abdominal pain

9. Cancer prevention:

9. 1. HPV Vaccines and Cervical Cancer:

Cervical cancer and sexual behaviour of population are directly proportional to each other, recent study shows that in the U. K HPV prevalence and possession increased consistently with increasing numbers of lifetime sexual partners, regular partners, and new partners in the last 5 years (Almonte, 2011).

The two prophylactic vaccines Cervarix and Gardasil consist of virus-like particles (VLPS), these are recombinant viral capsids made by expressing HPV 16 and 18 L1 proteins in insect cells through the baculovirous (cervarix) or HPV 6, 11, 16 and 18 L1 proteins in yeast cells (Gardasil) (Kahn 2005; Wang 2007; Kirnbauer et al., 1993). The virus-like particles (VLPS) contains

no viral DNA and therefore would not in any case cause an infection or cervical cancer in recipients (Wang, 2007).

Cervarix:

GlaxoSmithKline (GSK) produces cervarix vaccine; it is a bivalent containing HPV 16 and 18 L1 virus-like particle vaccines that works against HPV 16 and 18 infections and cervical cancer (Bayas et al. 2008; Govan 2008). A phase II study illustrated that Cervarix was 91. 6% efficacious against occasional infections and 100% effective against persistent infection (Harper et al. 2004). Cervarix is made up of an ASO4 adjuvant which contains aluminium hydroxide and 3-O-deacylated monophosporyl lipid (MPL), ASO4 helps improve the immune system (Bayas et al., 2008).

Gardasil:

Gardasil is developed by Merck and Co; it is a quadrivalent vaccine containing HPV 6, 11, 16 and 18 virus-like particles (Adams, et al., 2007; FDA, 2006). A phase II efficacy study of Gardasil results demonstrated that the vaccine has 90% efficacy in preventing incident HPV infection and cervaical cancer (Viller, et al., 2005). In June 2006 Gardasil was licensed by the FDA for use in young and adult females between the ages of 9 to 26 for the prevention of cervical cancer, genital warts and precancerous lesions (FDA, 2006), it was also approved in September 2008 for the prevention of vaginal and vulvar cancers caused by HPV 16 and 18 (FDA, 2008).

Both of the above vaccines are given in a series of three 0. 5ml immunisations over a time period of six months prior to a young person becomes sexually active (Long III, et al., 2007; WHO, 2007).

Figure 24 shows how the HPV DNA is detected in cervical cancer.

The above figure adopted from: Global Link (2008)

Date accessed 07/04/11

9. 2. Breast Cancer Vaccine:

Vaccine has been developed firstly against cervical cancer and now the scientists are trying to develop a vaccine against breast cancer, however scientists are trying to develop a vaccine which could be useful against all the different strains of HPV such as 16, 18, 33 worldwide (Armstrong, 2010).

Prognosis:

Table 14 shows the five year survival rates for colorectal and breast cancer.

(Howlader, et al., 2011).

There is only 23% survival rate for distant spread in breast cancer this shows that there is a need for more research to develop a vaccine against different strains of breast cancers and to treat these cancers affectively and avoid so many deaths.

10. Discussion:

Breast cancer is a malignant tumour that starts from cells of the breast. Cancer occurs due to mutations in the genes responsible for controlling the

growth of cells thus cells are unable to function properly (Sariego, 2010). Human papillomavirus (HPV) is considered as an aetiological agent for many cancers such as cervical cancer, breast cancer etc. High risk HPV types causes cancer by integrating into the host genome and causes disruption and loss of some of the viral genes such as L1 and L2 genes and also increases the expression of the early genes (Wang, 2007; Mera, 1997).

The aim of the research was to find out the association of HPV with breast cancer involving DNA extracted from archived breast tissue samples using DNA extraction method. This DNA sample could be amplified using PCR to find HPV genome specifically targeting E1 gene. This is conjunction with other studies in which samples were amplified using consensus primers CpI & CpIIG and targeted the E1 gene in a region conserved for 99% of most common HPV subtypes (Mendizabal, et al., 2008).

Given that the tissue samples were not fresh but were paraffin embedded which are not as good as fresh tissue samples because formalin fixation could denature the tissue during sectioning and also the DNA extracted from FFPE (formalin fixed paraffin embedded) tissues are usually at low concentration and disjointed (Shi, et al., 2006).

Additionally the experiment was carried out very successfully because most of the results that have been obtained were between the ranges of 1. 8 to 2. 0, which are regarded as pure DNA sample and therefore contains no protein contamination. A positive and negative control should be carried out while amplifying the DNA because a positive control makes sure the technique is working satisfactorily by using a reacting material relatively similar to the

test material and negative control tests the specificity of the reaction and ensures there are no false positives (Mendizabal, et al., 2008).

Although good results have been achieved however there were some variations in the purity of DNA extracted from the breast tissue samples and that depends on many different factors such as some tissue samples were darker in colour than normal which suggests the samples were not as fresh therefore it gave a lower result than the normal range of 1. 8 to 2. 0. Additionally the low results also depended on the way the whole experiment was carried out, there had been some mistakes in adding or mixing different solutions and mistakes were constantly recorded in the lab book therefore the same mistakes were not repeated again.

Moreover many different techniques have been learnt from this project including the usage of centrifuge, vortex, incubator and nanoviewer.

Carrying out this project has provided a full understanding on how to engage in the practical work which is beneficial in future laboratory projects, this is an ongoing work by the supervisors of the project to try and identify the association between the HPV and the breast cancer, if successful then a broader vaccine could be developed against all different strains of HPVs such as HPV16, 18 worldwide and to cure not only breast cancer but also many different types of cancers such as, cervical cancer, head and neck Squamous cell carcinoma, genital warts etc. This will reduce the amount of vaccination given to each patient and also it will have tremendous effect on the quality of life and will solve many problems and save many lives.

Furthermore many studies have been carried out to find out the presence of HPV in breast tissue. Some were successful by getting 86. 21% positivity of HPV infection in breast cancer (de Villers et al., 2005) this was in conjunction with other studies that have been successful in obtaining high positive result (Hening, et al., 1999; Gumus, et al., 2006; Kan, et al., 2006, Li, et al., 2002). Additionally according to a largest investigation on breast carcinoma specifically analysing mammoplasty and fibroadenoma specimens as a control group the authors were able to detect HPV DNA in 24. 5% of the breast carcinomas but were unable to detect any in benign breast specimens (Damin, et al., 2004).

However other authors have either achieved low positivity (Kroupis, et al., 2006; Tsai, et al., 2005) or HPV was totally absent (Lindel, et al., 2007; Gopalkrishna, et al., 1996). Therefore there are two different views on the association of HPV with breast cancer as it has been indicated by the above studies, which is normal because scientists can have different opinions sometimes.

This project was limited because only the DNA has been extracted and the DNA was not amplified by using PCR, in the future if this project were to be continued PCR can be used to amplify the gene from the DNA extracted in this project and the investigation can be expanded and more information can be obtained.

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