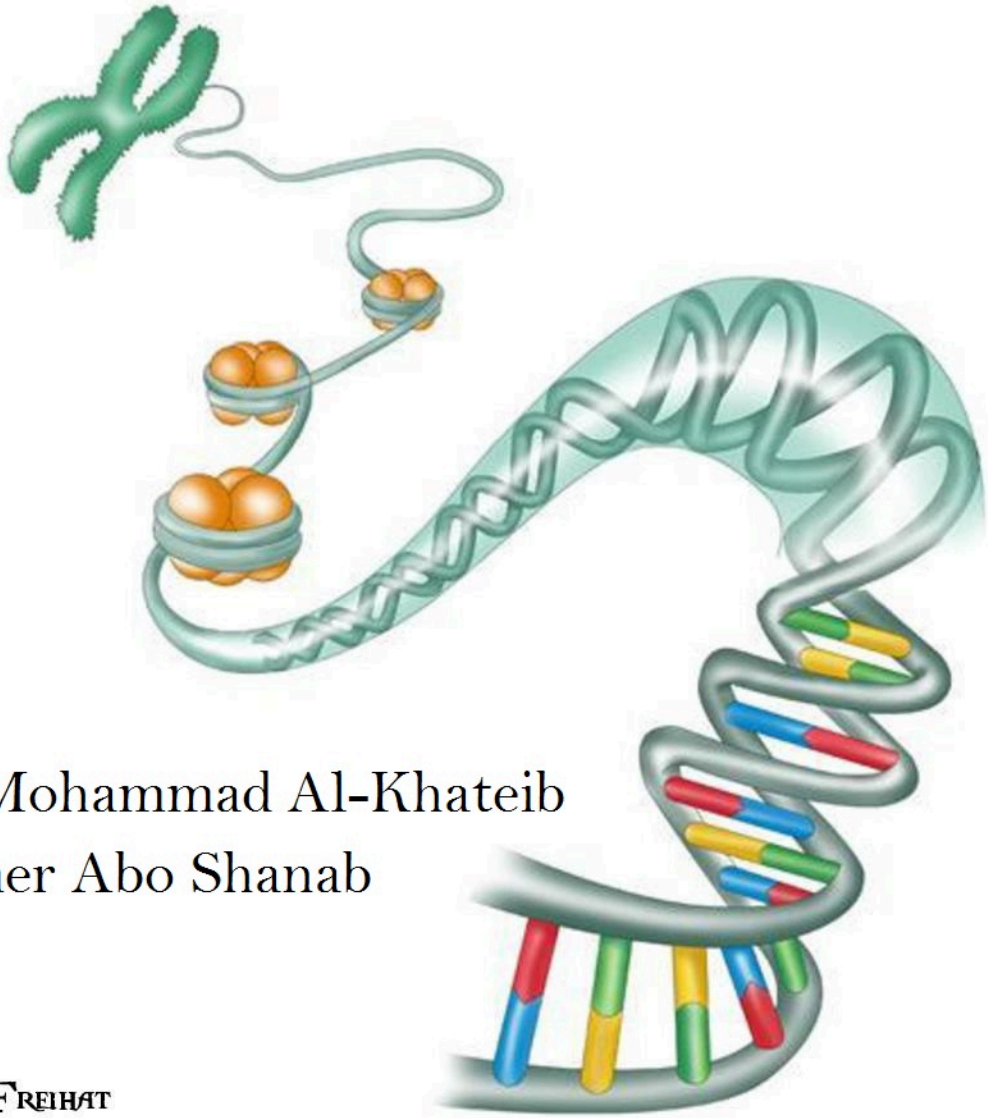
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

○ Slides ● Sheet ○ Handout ○ other.....



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بسم الله الرحمن الرحيم

Genetics Abnormalities (part 2)

Chromosomal abnormalities

هذه الشيت حاصلة على علامة الجودة ISO9001 مرتين من قبل

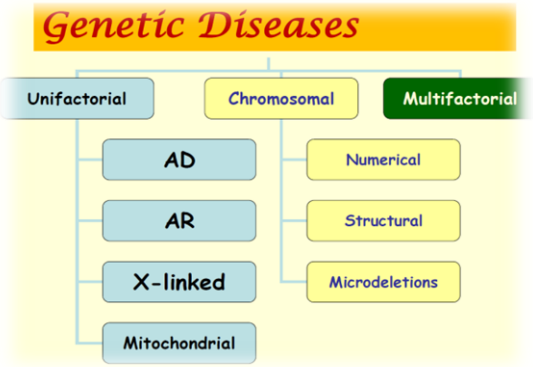
خالية من الالوان الصناعية , خالية من المنكهات الاصطناعية

تحتوي على جميع المعادن الاساسية لنمو طالب الطب (شيت و سلايد)

ملحوظة هامة : هذه الشيت تحتوي على مواد معدلة وراثيًا

Today we are ganna to talk about a part of genetic abnormalities , which is the chromosomal abnormalities, so we classify the genetic disease into 3 major component :

1- Uni-factorial (which means one abnormal gene = disease).
2- **Chromosomal abnormalities.**
3- Multi-factorial.



So today we will **talk about** the **chromosomal abnormalities** and how we can use this for **diagnosis** of genetic disease .

First of all , when we talk about chromosomes generally **we look to the** :
1- **structure** of the chromosome.
2- the **method of chromosome analysis** (how we can visualized the chromosomes and how we can examine them).

3- molecular **cytogenetics*** (how we can apply the molecular methods to diagnose these abnormalities).
4-chromosomes **abnormalities.**
5-chromosomes **nomenclatures** (how we classify them (giving names & discerptions to them))

***cytogenesis** = the study of **chromosomes number , structure , behavior & function** , in relation to gene inheritance , organization & expression .
***wiki** said that cytogenesis is the origin , formation & development of a single cell .(say hello to wiki)

So chromosomes as we know they are generated during cell division , and the **cell division has 4 stages** :
(1- **G1** phase 2- **S** phase 3- G2 phase 4- **M phase (mitosis phase**))

M phase (mitosis) is 4 stages : (very imp. In this lecture)

(1-interphase 2-prophase 3-metaphase 4-anaphase & telophase)

During M phase the chromosomes of somatic cell would segregate from each other to 2 different (daughter) cells (4n = 2* 2n) . These daughter cells theoretically should be identical , as well as they should contain the same number of chromosomes .

2 types of division :

1-**somatic cells** do → mitosis → the daughter cells contain the same # of chromosomes of parents (one 46 ch. cell Gives 2* 46 ch. Cells)

2-**germ cells** do → meiosis → the daughter cells contain the 1\2 # of chromosomes of parents (one 46 ch. cell Gives 2* 23ch. Cells)

Since the both the sperm & the ovum contain 23 chromosomes then after fertilization the fetus (46, xx or 46, xy)

Now after we have this fertilization (46 ch.) we start testing (studying) these chromosomes, if we are talking about **inheritance abnormality** this mean that the **problem was in the sperm (or/and the ovum)**, so the result is **genetic disease** (anything (any problem) happens before birth would produce genetic disease) , **while anything (any problem) happen after birth would produce a nongenetic problem** .

So when we talk about cytogenetic (cytogenesis) we are talking about : the number, structure, function & the behavior of the chromosomes, and also we are talking about how these chromosomes are organized in the cell and how they are expressed .

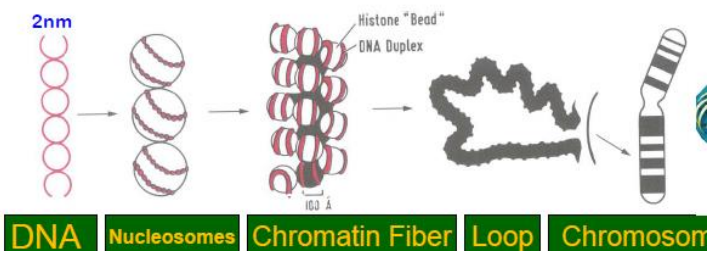
During division each one of these **chromosomes**, in the somatic cells, are **divided into 2 chromatid**, then each chromatid would move to one of the daughter cells, so the daughter cell would contain 46 chromatid, then these chromatids **(46 chromosomes → 92 (46+46 chromatid after separation) → 46 chromatid in the first daughter cell (and other 46 in the second daughter cell) → replication of these 46 chromatid in each daughter cell → 46 chromosomes in each daughter cell)**

The structure of the chromosomes :

The **chromosome** is consist **of double-helix DNA** , this DNA is **coiled around histones to form nucleosomes**, these nucleosomes are **coiled around themselves to form chromatin fiber**, this fiber would **coiled around itself to form a loop**, this loop is a very small segment in the chromosome .

This chromosome is a very condense band found inside the nucleus of the cell .

All the 46 chromosomes in the



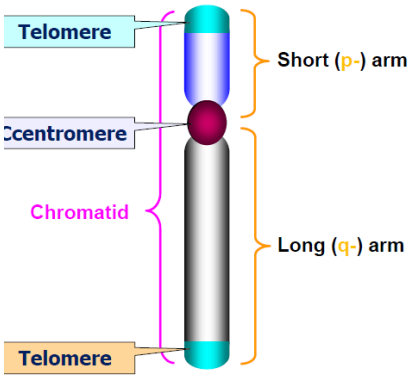
somatic cell contain about 3 billion nucleotides (all inside one cell)
سبحانك ربي

So these **chromosomes morphologically** consist of certain parts;

1-the **centromere** (the constriction in the middle of the chromosome)

2-**p-arm** (the upper short arm) ----------> **p-arm with upper (p with p)**

3-**q-arm** (the lower long arm)



The chromosome consists of 2 sister (identical) chromatid (as we said) , and those 2 are connected to each other by what?! By the centromere ☺

At each end of each chromatid there is the **telomere**, which is a very specific structure which **consists of repetitive nucleotides (6 nucleotides = TTAGGG)** , the # of these repletion is variable according to the age of the person :

1-**at birth** → there are about **8 thousand** repetitions

2-at **35y old** → there are about **3 thousand** repetitions

2-at **65y old** → there are about **1.5-2 thousand** repetitions

Now **the function of these telomeres is to keep the integrity of the chromosome** (the chromatid), because the chromosomes are getting shorten after each replication, and these **telomere scarifies** by its own nucleotide in order **not** to lose a functional nucleotide, (there is an enzyme called **telomerase that can add repetitive TTAGGG to the end of the telomere in order to save it**).

Visualization , isolation and examination of chromosomes :

All human cells contain chromosomes, so , if we want to test a chromosome what is the best cell to take its chromosome ?

The **lymphocyte**, because it is **ease to be isolated from the peripheral blood** as well as it is **ease to induce its replication** (division**) by** using **Phytohemaggultinin**.

Now we can visualize the cells by :

Take a blood sample from the patient, then cultured this blood in a culture media that contains all the substances necessary for the growth of the cells, then we add Phytohemaggultinin to stimulate these division of these cells, after about 3 days (72 hours) at 37 C we **stop the division** of these cells **by anticancer drugs (called colcemid**) which would stop its division **at the metaphase of the M stage** (do u remember it)

So do u ask ur self why at the metaphase ?!

Because the chromosomes are already segregated, and moving on to the poles of the cell , and this is what we need to see under the microscope , a segregated chromosomes (far from each other) ☺

After 2 hours of adding colcemid we isolate the chromosome, then we put them **in a tube contain RBCs & hypotonic solution** (to make the fluid go inside the cells and these cell then would swell → by this **swelling the chromosomes would segregated in a better way**)

Then we **fix these cells at this stage on a slide** (with a specific stain) and finally put them under the microscope (we can manipulate these chromosomes at this stage by many methods to find and test what we want).

Now we examine the chromosomes under the microscope in 3 different ways:

1- G-banding ("**banding**" is discussed below).

2- **Hypridization using Fluorescence.**

3- **Using Molecular technique**

A- G-banding :

After using "banding" ways (discussed below) , the chromosomes is clear to us , and we look for one of **the following characteristics** :

1-the **size** of the chromosomes.

2-the **location of the centromere**.

3-the **pattern of the bands in the chromosome** (discussed below).

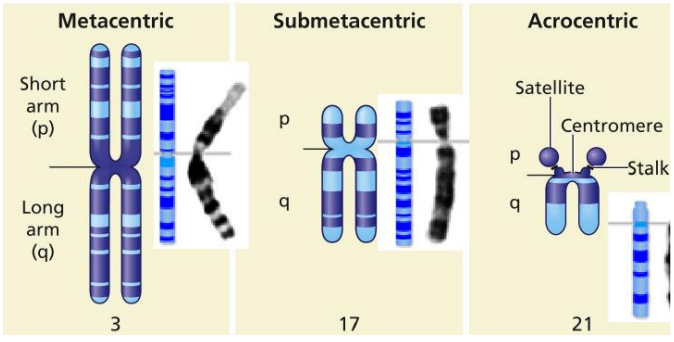
Explanation of these characteristics:

1- The location of the centromere :

We can classify the chromosomes into 3 types depending on the centromere location :

a- **Metacentric** : which means the location of the centromere is almost in the middle (**here we find that p-arm = q-arm**)

b **-submetacentric**: which means that the location of the centromere is in the upper part (**here we find that p-arm < q-arm)**



c- **Acrocentric** : which mean that there is **NO p-arm (only q-arm)**

2- The pattern of the bands in the chromosome :

We applied on the slide what we call "banding"

Banding: is a proteolytic enzyme that can degrade the histones (as a result the nucleosomes) so at **this area the DNA would be free →**

the remaining of the chromosomes are not stained in an even way; one part would dense and other part would be light (opened), this alternating staining of dark and opened areas is called banding.

The # of bands in the total chromosomes is known and its about 450 bands in 64 chromosomes (in low resolution banding).

We have different types of banding :

1-C-banding (centromere banding → to look for the centromere)

2-Q-banding

3-R-banding (reverse banding)

4-G-banding

CQRGe سكرجي (هاظ الي طلع معي عاد) (: (:

We use these different banding types in order to clarify a certain band(area) on a certain chromosome, which (that area) was NOT clear using another type of banding , take this eg. , if we did G-banding on a chromosome and the area around the centromere appeared dark , then we use the R-banding so the same area would appear light (clear) .

Now what is the difference between the dark & the light band?

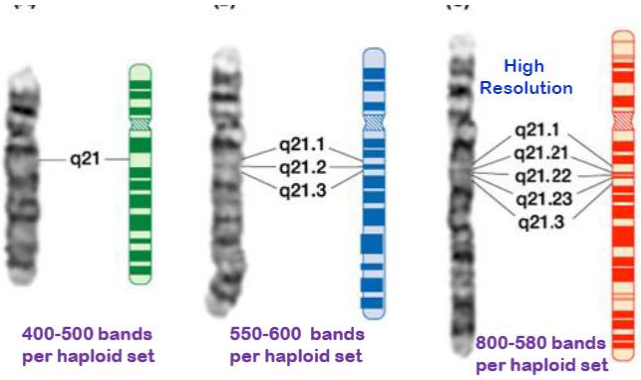
In the **light band** we can see a **replication section** in the **S phase ,** where the chromosome started its replication, **less condense**, **transcriptionally active** , and **rich in CG** nucleotides.

On the other hand , the **dark band** is the opposite , so its **replicate later , more condense , transcriptionally inactive**, and **rich in AT nucleotides.**

The third type of bands , which is the **centromere band**, is **to join the sister chromatids together**, and it's about hundreds of kb of repetitive DNA, sometimes we can find two centromeres (dicentromeres) in the same chromosome in certain abnormalities.

The last type of bands could be seen is **the telomere band** , which is a **DNA and protein cap** , ensures replication to tip , tether to nuclear membrane , and provide terminal stability to the chromosomes to ensure its survival .

Now , what if , I couldn't see (or compare) what I need in that chromosome ?!

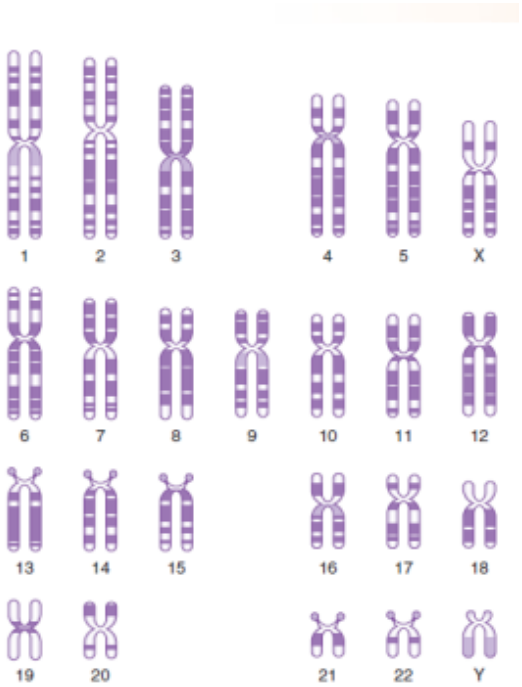


In this case we **use what we call the HRGb (high resolution G banding) ,** but **in this case the chromosome should be longer (to get more bands**), **so we stop the cell division at the prometaphase (NOT in the metaphase**) where the chromosomes are longer and thinner. After we do the **HRGb, It will end up with 800-850 bands** (NOT 450 like in the low resolution banding).

For eg. The 7q21 band in the low resolution would appear as 7q21.1 & 7q21.2 & 7q21.3 in the HRGb ..... while the 7q21,1 in a more higher resolution would appear as 7q21.11 & 7q21.12 & 7q21.13

We put the results on a specialize computer, which would group the chromosomes into groups, then we can examine them to see if they are normal or NOT.

Let's **go back to the chromosomes**, we said that the human body cell **contain 46 chromosomes** , because each 2 come together so we end up with **23 pairs (22 autosomes (somatic) + 1 sex ch. pair (germ = xx or xy))**, so **<u>we classify these chromosomes pairs into 7 groups according to the previous characteristics:</u>**



1-A-group (largest , metacentric→chromosomes #1,2,3)

2-B-group (large , submetacentric→ 4,5 +x)

3-C-group (medium , submetacntric →6,7,8,9,10,11,12)

4-D-group (medium, acrocentric → 13,14,15)

5-E-group (small, submetacentric →16,17,18)

6-F-group (smallest, metacentric →19,20)

7-G-group (smallest, acrocentric → 21,22 +y)

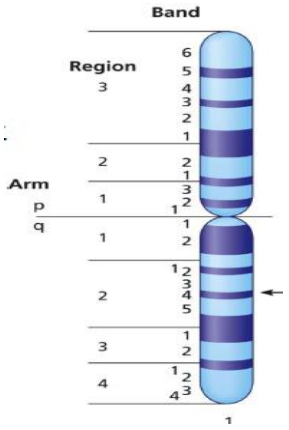
- A 1-3
- B 4-5 + X
- C 6-12
- D 13-15
- E 16-18
- F 19-20
- G 21-22 +Y

After knowing all of that , how we can **specify the location of a mutation on a chromosome?**!

By using the ISCN system ,

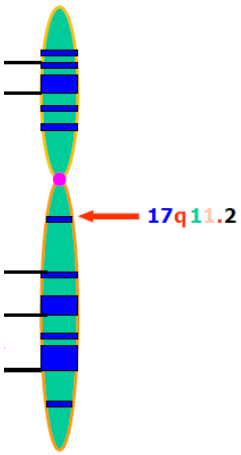
What is the ISCN system (international system for human cytogenetic nomenclature) ?!

The **ISCN is a way to nomenclature (naming) of the sites (areas) on a single human chromosome, so each chromosome has its own ISCN**.



For eg. If we took chromosome #1 we will find that there is 2 arms (q&p arms), (3 region in the p-arm from the centromere)&(4 region in the q-arm from the centromere), and further divided into bands and sub-bands , like , if someone ask me to identify (1p31.1) site Im going to say that :

Chromosome # 1 (1), short upper arm (p) , region #3 (3) , band #1 (1) , sub-band #1 (.1) → 1p31.1



So we walk like this : chromosome# → arm latter → region# → band # → sub-band # .

Now try to identify the the site in

the pic. At the right (the 17q11.2 site)

So , let's go further deep , **<u>if we want to identify a certain mutation location in a person , what should we do ?!</u>**

Simply , follow me on twitter , No No I mean follow this equation →

(#of ch. , sex ch. , type of the mutation+its location)

<u>Lets take tow eg, :</u>

1- 46,xx,del(5p)

46 = #of ch. , xx = sex ch. , del(5p) = type & location of the abnormality

Del(5p) mean deletion mutation at chromosome #5 at the end of (p-arm)

2- 46,xx,t(2;4)(q21;q21)

46 = #of ch. , xx = sex ch. , t(2;4)(q21;q21) = translocation mutation between chromosomes #2 & #4 at the q-arm (we put between them ;) 2nd region , 1st band of both of the chromosomes (we put ; between the arm letters and region, bands and subbands numbers of the two chromosome)

Types of the abnormalities :

Normally: Male = 46,xy ……. Female = 46,xx

B- Hypridization using Fluorescence: fluorescence in situ hybridization (FISH سمك)

After we count the chromosomes, banding them, and looking for any abnormality, if I couldn't find anything but the patient still show a clinical picture and there is certain chromosome suspected to cause the abnormality then we turn to this method.

The procedure :

1- Make complementary segments for many part of the DNA that could be affected (mutated) → so if the normal DNA contain (TAGAT**T**CT) while the mutated one contain (TAGAT**C**CT) then I would make a probe for the mutated one contain (ATCTAGGA)
2- Then we label these segments by fluorescence pigment
3- Then we add the chromosome that we want to test to the tube which contain that probes.
4- After that we heat the tube → the DNA double strand would separate from each other
5- Then we cool it , so I would have one of the following :

a- If the person have the disease (the mutation)

ISCN

- del - deletion
- dic - dicentric
- fra - fragile site
- i - isochromosome
- inv - inversion
- p - short arm
- r - ring
- der - derivative
- dup - duplication
- h - heterochromatin
- ins - insertion
- mat - maternal origin
- Pat - paternal origin
- q - long arm
- t - translocation

then the fluorascenated probe would attach to the DNA → colorful DNA.

b- If the person don't have the mutation → the old 2 strand would go back to each other → NO hybridization →NO colorful DNA

*we should mention that :

In the "banding" we stop the cell division at the metaphase (or sub-metaphase in HRGb) , **while in the FISH we stop the division either at the interphase or the metaphase**, and here is the advantages of each one of them :

1- Stopping at the interphase :

Don't need culturing nor stimulating , 200-500 cells are enough for FISH

2- **Stopping at the metaphase** :

We use it when the probe we produced bind (covers) the whole chromosome (good for small rearrangement which is NOT detected by "banding") , and also **for detection of the telomere or centromere mutation .**

Note : the applications and the SKY in the slides didn't mentioned in both sections

c- Molecular technique : (CGH (comparative genomic hybridization))

If we tried the previous methods and no one work , then we turn to this method.

In this method we do hybridization for the whole 46 chromosomes (NOT for only one like in FISH), and **here is the procedure** :

1- We take 46 normal chromosomes (from a 100% normal cell) and color them with a red color (46 chromosomes as 23 pairs).

2- We take 46 chromosomes from the suspected tissue (from a cell which we suspect that there is an abnormality (mutation) in it) and color them with a green color (46 chromosomes as 23 pairs).

3-we put all the 92 chromosomes (46 pairs) together in one tube

4-we heat the tube → separation of all the chromosomes from each other

5-then we cool the tube, and I will end up with one of the following :

a- If the suspected chromosomes is exactly similar to the 100% normal chromosomes then each one of the 46 green chromosomes would beattached to the complementary one of the 46 red chromosomes so I **will get 46 orang pairs of chromosomes → NO problem → Nothing wrong**

b- If the suspected chromosomes is NOT exactly similar to the 100% normal chromosomes and there is extra chromosomes (duplication) in the suspected chromosomes then each one of the green chromosomes would be attached to the complementary one of the red chromosomes so **I will get a number of orang pairs of chromosomes with some green-only chromosomes (the extra ones) → extra green → problem.**

c- If the suspected chromosomes is NOT exactly similar to the 100% normal chromosomes and there is some missed chromosomes (deletion) in the suspected chromosomes then each one of the green chromosomes would be attached to the complementary one of the red chromosomes so I will get a number of orang pairs of chromosomes with some red-only chromosomes (those normal ones that didn't found its complementary) → extra red → problem.

اهداء :

1- مصطفى عيسى – صاحب القلب الحنون ☺ ☺

2-عبد الله شفاقوج – نعيماً

3-ليث علاونة – جبتلي العيد

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5-يوسف توبة – قدها

6-عبد الله حوري – واي نت

Special dedication to Amer ABU Shanab & R

وآخر دعوانا ان الحمد لله رب العالمين